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EARLY HISTORY, IN PART ESOTERIC, OF THE HOOKWORM (UNCINARIASIS) CAMPAIGN IN OUR SOUTHERN UNITED STATES

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INTRODUCTION

Recent literature contains several accounts of the origin of the hookworm campaign in our Southern States. As some of the data have been presented by authors who were not connected with the movement, it is not unnatural that discrepancies have crept into the publications.

Because of my intimate connection with the early history of the movement, repeated requests have been made of me to supplement the existing accounts, paying particular attention to the earlier years and (for the sake of historic accuracy) clearing up certain points of discrepancy. The chief reason why these requests have not thus far had results is my antipathy to using the perpendicular pronoun. In now complying with the requests, humble apology is herewith presented for using the first person singular so frequently; but unfortunately this technique can not be avoided entirely. The important points are *a* that the campaign took place, *b* that tens of thousands of patients were treated, and *c* that preventive measures have been instituted. The question as to who did what, when and why, is of infinitely secondary interest.

In order to follow the subject clearly it is well to keep in mind certain data on terminology and nomenclature.

TERMINOLOGY

The *cutaneous* (initial) stage of hookworm disease is known under various terms as follows:

Ankylostomum dermatitis, chauffie, cow-lot itch, foot-itch, ground-itch, larva migrans (in part), mazamorra, new sump bunches or blotches, pani-ghao, sore feet

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of coolies, toe-itch, uncinarial dermatitis, water-itch, water-pox, water-sores, wet weather-itch, etc.

This condition has been recognized for many years, but it was not known until the brilliant investigations by Looss (1898 b; 1901 h, i; 1903 d) that this represents the initial stage of hookworm disease. In our Southern States, the rural laity in general has long been familiar with "ground-itch," but occasionally confuses it with other conditions.

The *later* stage of the disease is known under many terms, as for instance:

Uncinariasis, "AAA" (Eber's Papyrus), Aegyptische Anämie, Aegyptische Chlorose, Agchylostomiasis, Allotriophagia, Alotrofagia, Amarellão (Gelbsucht, Brazil), Anaemia intertropicalis, Anemia of coolies, Anchilostomanemia, Anchilostomi, Anchilostomia, Anchilostomiasi, Anchilostomoanemia, Anchilostomosi, Anchylostoma-anaemia, Anchylostomen-Krankheit, Anchylostomiase, Anchylostomiasis, Ancylostomen-Anämie, Ancylostomiasis, Ancylostomose, Anemia da anchilostoma, Anemia d'Anzin, Anemia de Ceilán, Anemia del Gottardo, Anemia del minatori, Anemia de los mineros, Anemia montana, Anemia dei solfatari, Anémie ankylostomique, Anémie des houilleurs, Anémie des mineurs, Anémie des pays chauds, Anémie du Saint-Gothard, Anémie intestinale, Anémie tropicale, Ankylostoma-Anémie, Ankylostoma infection, Ankylostoma-Krankheit, Ankylostomasie, Ankylostome infection, Ankylostomiase, Ankylostomiasis, Ankylostomo-Anaemie, Anquilostomiasis, Atrophia a ventriculo, Bergmannsanämie, Beri-beri (Ceylon, in part), Bleichsucht, Cachexia Africana, Cachexia Americana, Cachexia montana, Cachexie africaine, Cachexie aqueuse, Cachexie aqueuse de l'homme, Cançaçao (Brazil), Cançaço, Canguary, Cansancio (Costa Rica), Cansera (Nicaragua), Caquexia acuosa (Antilles), Chloroanemia, Chlorose d'Egypte, Chnotophagia, Chthonophagie, Cotton mill anemia, Dirteating [in part], Dissolution, Dochmiasis, Dochmiose, Egyptian chlorosis, Empoisonnement volontaire [of slaves], Enfermedad de microbio (Costa Rica), Epidemic anemia, Erdessen, Gastroentérite chronique des nègres, Geofagia, Geophagia, Geophagie, Grubenwurmkrankheit, Herzklopfen, Herzweh, Hookworm anemia, Hookworm disease, Hydrhémie vermineuse, Hypoemia intertropical, Hypoemia tropical, Hypohaemie, Hypohemie, Langue blanche, Magenweh, Malarial anemia [in part only], Malaria-Chlorose, Malattia dei minatori, Mal-coeur, Mal de coeur, Mal d'estomac, Mal d'estomac des nègres, Mal de Estómago de negros (Antilles), Malnutrition [in part only], Mineur Anämie, Necatoriasis, Negro cachexy, Negro consumption, Opilation, Oppilaçao, Palpitations du coeur, parasitäre Anämie, Pica, Portuguese white liver, St. Gothard Tunnel disease, Safura, Selbstvergiftung [of slaves], Sumph- Cachexie, Tetum, Tropical anemia, Tropical chlorosis, Tropische Chlorose, Tropische Hypoämie, Tunnel worm disease, Tuntun, "UHA" (Eber's Papyrus), Uncinaria disease, Uncinaria infection, Uncinarial infection, Uncinariasis duodenalis, Uncinariose, Uncinariosis, Weisse Zunge, Wurmkrankheit, Ziegelbrenneranämie, etc.

It will be noticed that a number of these synonyms and variants are based upon symptoms; historically some of them came into being prior to the discovery of the etiological factor (hookworms) or at least before the general acceptance of hookworms as the cause. Under these circumstances, earlier literature based on symptoms must be correlated cautiously with more recent literature in which the etiological factor is definitely mentioned. This process is rather common in medicine: for instance, compare tuberculosis, leprosy, diphtheria, typhus, gonorrhea, syphilis, etc., recognized clinically long before the causative factors were known.

NOMENCLATURE

There are three species of hookworms known for man, and a fourth species has been reported at least once. Some authors speak of these as "varieties," but from the present systematic point of view these parasites represent distinct "species" belonging to two "genera":

Genus *Ancylostoma* Dubini, 1843, type species *A. duodenale,* with hooks in the mouth, and

Genus *Necator* Stiles, 1903, type species *N. americanus,* with semilunar plates instead of hooks (also with certain additional anatomical differential characters).

These two type species (*A. duodenale* and *N. americanus*) are the important hookworms of man. A third species (*A. braziliense*), of dogs and cats, occasionally infects man, causing especially one form of *larva migrans;* the fourth species (*A. caninum*) of dogs is from present data a very rare parasite of man.

The original genus of hookworms was known as *Uncinaria* Froelich, 1789. Both *A. duodenale* and *N. americanus* were for a time classified under this generic name, but as anatomical studies advanced and as systematists changed from "lumpers" (who accepted broad genera) to "splitters" (who accepted narrower genera), *Ancylostoma* and *Necator* became generally recognized as distinct genera. This was simply a process of evolution in conception of generic limits and is quite general in systematic zoology. An intermediate stage in the process is the recognition of sub-genera.

REPORTS OF HOOKWORM DISEASE IN THE UNITED STATES AND IN PUERTO RICO PRIOR TO 1884

Hirsch's (1883 b) Handbuch der historisch-geographischen Pathologie marks the beginning of an important period. Prior to the appearance of this work, all references to the disease in our country and in Puerto Rico mentioned the malady under various designations based largely or entirely upon prominent symptoms without reference to the parasites.

Chabert, 1821 a: 188–193. Dirt-eating; Louisiana.
Cotting, 1836 a: 288. Clay-eating; Georgia.
Duncan, 1850 a: 194–195. Dirt-eating; anemia; Louisiana.
Ely, 1868: 101, 102. Chalk-eating: Cause?
Gould, 1876 a: 417. Pica; New Hampshire: Cause?
Heusinger, 1852 a: 19. Puerto Rico.
Jordan, 1832 a: 18–30. Cachexia africana.
Le Conte, 1845 a: 415. Dirt-eating; Georgia.
Letherman in Coolidge (1856: 333). Dirt-eaters not uncommon near Ft. Meade, Florida. "The common remedy is said to be whisky or cider in which nails have been steeped."
Little, 1845 a: 70. Dirt-eating; Florida.
Pitt, 1808 a: 337. Dirt-eating; North Carolina.

Pollard, 1852 a: 185. Dirt-eating; Richmond, Virginia.

De Cordoba (1831) in his four volume work on Puerto Rico devotes a chapter in Volume I to the diseases on the island and on page 204 mentions briefly mal de estómago (later, 1883, recognized as a synonym for hookworm disease) as follows:

"Entre los negros reynan dos enfermedades particulares, que son la que llaman Pian y el mal de estómago: los efectos de esta última son mudar la cùtis en un color de azeituna, la lengua blanca, un sueño profundo, con una languidez, que les imposibilita para el menor exercicio: toda la maquina se aniquila y destruye; el disgusto de los alimentos dulces y saludablas, acompaña à la pasion por todo lo salado y picante; las piernas se les hinchan, el pecho se les levanta, y son pocos los que escapan, la mayor parte mueren sofocados despues de haber sufrido muchos meses."—Memor. Georgr. [etc.] de la Isla de Puerto Rico. Sanmiltan. Vol. 1, p. 204.

Heusinger (1852: 19) quotes these observations by de Cordoba.

Hirsch (1883 b: 1885 a: 1888 a) in his scholarly treatise brought the geographical-medical literature together under the heading *Anchylostoma duodenale.* He says:

[p. 313] "At the beginning of the 18th century the attention of French and English observers was drawn to a very destructive form of disease prevalent among the negroes of the West Indies and Guiana. * * * The same malady was afterwards met with * * * at other parts of the globe—in Brazil, Egypt, the West Coast of Africa, and elsewhere * * * [p. 314] it has been variously named as follows: mal d'estomac, mal de coeur, dirt-eating, hypohaemia or anaemia intertropicalis, oppilaçao or conçaço (Brazilian), cachexia Africana, cachexie aqueuse (French).

"The earliest accounts of this disease are to be found in the narrative of travels by Père Labat (Nouv. voyage aux isles de l'Amérique, Par., 1742, ii: 11.) of Guadeloupe, and in the history of the British colonies in the West Indies by Bryon Edwards [1793] * * * and attributed most of the deaths among the negroes to two diseases,—trismus nascentium and 'mal d'estomac.' Then follow numerous scientific reports * * * by English, French and Danish practitioners of the last and present centuries who had had opportunities of observing the malady on several of the West Indian Islands (Hunter [1833], Mason [1833], Telford, Gregory and Ferguson for Jamaica; Desportes and Chevalier [1752] for Domingo; de Cordoba [1831] for Puerto Rico; Dons for St. Thomas; Grall for St. Martin; Moreau de Jonnés [1816] and Duchassaing for Guadeloupe; Savaresy, Noverre [1833], Carpentin and Rufz for Martinique; Imray [1843] for Dominica; Levacher [1834] for Sta. Lucia; Chisholm for Grenada; McCabe for Trinidad.) * * * v. Leent [(l.c., 1880 a) for Surinams.] * * * and, if only to a slight extent, among the negroes in some parts of the Southern States of the American Union (Chabert and Duncan for Louisiana; Lyell for Alabama and Georgia). The accounts cited by Heusinger ('Die sogenannte Geophagie, &c.', Cassel, 1852[1]) of Geddings for South Carolina, as well as the statements of Little (Am. J. Med. Sc., July 1845, p. 70) and Letherman (in Coolidge's 'Report on the U. S. Army, &c.', Washington, 1856, p. 333), both from Florida, relate undoubtedly to the malarial cachexia."—Hirsch, 1885 a, **2**: 213–214 (translation of 1883 b: 218–219); see also 1888 a.

Although several authors had already recognized the parasitic nature of the disease in Europe, Africa, in the Antilles and in South America, no evidence seems to be available that hookworms had been reported for any patient in Puerto Rico or in the United States. Thus Hirsch's report of hookworm disease for this Spanish island and for our Southern States was based upon an interpretation of symptoms and of designations, simi-

[1] This book contains an extensive discussion of "dirt-eating" with many references to this condition in the West Indies.—C.W.S.

lar to the cases of tuberculosis and other diseases at a time prior to the local recognition of the etiological organism.

R. Blanchard (1888 a) seems to be the next author who included our Southern States and Puerto Rico within the hookworm disease area. He says:

"En certains pays d'Amérique, l'Ankylostome atteint une telle fréquence qu'il est au nombre des plus grands dangers que puisse encourir la population; nulle part il n'est plus abondant qu'aux Antilles et au Bresil. Aux Antilles, il cause la cachexie aqueuse, le mal-coeur, le mal d'estomac des négres, la chlorose tropicale, maladie qui a été observée tout d'abord à la Guadeloupe, en 1742, par le P. Labat, puis à la Jamaique, en 1793, par Bryon Edwards. Depuis lors, on l'a constatée tour à tour à Saint-Domingue, à Porto Rico, à Saint-Thomas, à Saint-Martin, à la Martinique, à la Dominique, à Sainte-Lucie, à Grenade, à la Trinité, etc. Elle s'observe encore dans les Guyanes. Bajon et Segond l'ont constatée à Cayenne, où Riou Kérangal a vu le parasite; Rodschied et Hancock l'ont observée dans la Guyane anglaise; Cragin, Hille, van Leent et d'autres ont noté son existence à Surinam."

"L'ankylostomasie s'observe encore en d'autres régions d'Amérique. Aux États-Unis, Chabert et Duncan l'ont rencontrée dans la Louisiane; Lyell l'a constatée dans l'Alabama et la Georgie; les observations d'Heusinger et de Geddings pour la Caroline du sud, celles de Little et de Letherman pour la Floride sont douteuses et se rapportent plutôt à la malaria."—Blanchard, 1888 a: 768–770.

Lutz (1885 c: 2319) reports this disease as very wide-spread on Greater and Lesser Antilles, and (1888 l: 157) refers to ankylostomiasis in the United States (Alabama, Georgia, and Louisiana), basing his statements on Hirsch's book.

Railliet (1893 a: 470) cites hookworm disease for the Antilles.

Scheube (1896 a: 319), on basis of literature, reported hookworm disease as follows:

". . . in Amerika einige südliche Staaten der Union (Louisiana, Alabama, Georgia, Süd-Carolina und Florida), die Antillen, Columbia, Venezuela, Guyana, Brasilien, Peru und Bolivia, ferner Queensland und die Fidji und Sandwish-Iseln."

Later (1902 hh: 207–219; 1903 k (1): 32) I did not hesitate to interpret certain early records, from Pitt (1808) to Duncan (1850) as referring "in many, if not all, of the cases to" hookworm infection.

CASES REPORTED FROM 1884 TO 1899

Blickhahn (1893 a) appears to be the first author definitely to publish the presence of hookworms in a patient in this country (at St. Louis, Mo.), and Moehlau (1897 a) followed with a report of five cases at Buffalo. Both authors attributed their cases to the "Old World" hookworm (*A. duodenale*). It should be added however that Herff (1894 a) records a supposed [and probable] case observed in Texas in 1864; and that Allen J. Smith (published by Schaefer in 1901 a) found hookworm eggs in feces of a man in Texas in 1895.

From the foregoing data it is clear that the presence of hookworm disease in the United States and in the Spanish island of Puerto Rico was a matter of professional record as early as 1883 (based on symptoms and

synonyms) and that at least six cases were recorded (1893, 1897) in the United States in which hookworms were definitely recognized as the etiological factor.

In the meantime, hookworm disease was recognized in the West Indies and Texas on basis of symptoms and (or) confirmed by finding the parasites, by several authors, for instance:

Pereira (1877 a: 25). Gives distribution as 40 degrees N. to 30 degrees S.

Lombard (1879 a: 413). Antilles, mal de coeur.

Rey (1880 a: 514). Cites *A. duodenale* for the Antilles (la Martinique, St. Thomas, La Dominque), [etc.].

Strachan (1885 a: 1291–1292). *A. duodenale* is frequent in Jamaica.

Lutz (1885 c: 2318–2319). Very wide-spread in the Greater and the Lesser Antilles; (1888 1: 122) very frequent in the Antilles.

Rake (1888 b: 111). Reports *A. duodenale* for Trinidad.

White (1888: 111). Reports an autopsy (1887) in Trinidad, with "swarms of *A. duodenale.*"

De Brun (1893: 23). Antilles.

Herff (1894 a: 615). Small worms found (1864) in autopsy of a Mexican woman are now (1894) interpreted as hookworms; several Italians with similar symptoms.

Galgey (1897: 200–202). Refers to fully 85 cases of *A. duodenale* at St. Lucia treated with Thymol. See also Brit. Med. J., Jan. 23, 1897, 1: 222. St. Lucia. Medical men about to commence practice in tropical countries ought to familiarize themselves with what is known in the subject [ankylostomiasis]; otherwise they are likely to waste iron and arsenic in vain attempts to cure anemias, which a little skill in diagnosis and a few drops of thymol might dissipate in a week.

Boyd (1898: 53). In discussing ankylostomiasis in the West Indies, states that * * * the parasite * * * is known for * * * "Trinidad, Jamaica, and Grenada."

Mapleton (1898: 249–250). Reports ankylostomiasis for the Leeward Islands; thymol.

Zinn & Jacoby (1898 c: 49–51). Cite *A. duodenale* for St. Lucia, Trinidad, and Jamaica, on basis of literature.

McDonald (1899 a, 1900 a). West Indies, Antigua, Leeward Islands; 148 cases (34 fatal).

My first interest in this infection dates back to student days under Leuckart at Leipzig. Upon returning home to enter government service (July 1, 1891) in the U. S. Bureau of Animal Industry, almost the first thing I did was to examine the collection of parasites, in the Bureau, assembled by Cooper Curtice and Albert Hassall. In this study I was surprised not to find hookworm specimens from man despite the number of bottles containing hookworms from dogs, cattle, and sheep. The presence of this group of parasites in North American domesticated animals seemed to indicate that conditions in this country were favorable to the development of allied parasites in man; in addition, literature reporting cases of hookworm disease of man for the United States, the West Indies, and Central and South America; further the fact that many of our immigrants had come from parts of Europe, Asia, and Africa in which hookworm disease in man was known to occur, persuaded me that this malady must surely exist here more frequently than reported, but that it had been rather generally overlooked. This view was expressed in

the first course on this subject given in the Johns Hopkins Medical School, and my good friend Dr. William Osler, who was present, took me roundly to task for what I said. He maintained that a disease, as easily recognized as is hookworm disease, could not be generally overlooked in case it were present. In every lecture (1892–1897) on this malady before the students of Georgetown, Johns Hopkins, and the Army Medical Schools, I made it a point to repeat the remark:

"Gentlemen, if you find cases of anemia in man in the tropics or subtropics, the cause of which is not clear to you, consider the possibility of hookworm disease; make a microscopic examination of the feces and look for the eggs."

Early in 1898, I was ordered to sail immediately for Germany "to be gone for from three months to three years," to serve as Scientific Attaché at the United States Embassy in Berlin. Before our ship made port in Europe, the Spanish-American war had begun. For about two years I was unable to follow the subject further except in correspondence.

Tebault (1899 a, b) recorded a case of hookworm disease in a boy in New Orleans.

CASES PUBLISHED 1900 TO 1902

Upon returning to duty in Washington, I learned that Ashford had "in early January, 1900" called at my laboratory with a bottle of worms he had collected in Puerto Rico and which he had determined as *A. duodenale,* but which he did not leave for me to examine. He talked with Albert Hassall, my assistant, to whom he stated that he had found hookworm disease common in Puerto Rico. The next time Ashford and I met (during a later call at my laboratory), he described in detail his observations on the island and received the well-deserved hearty congratulations.

Upon being asked how he came to examine for hookworms he replied that upon failing to find malaria or other satisfactory explanation of the local anemia, and upon noticing the high eosinophilia, he recalled that in my lectures, which he had attended both in Georgetown Medical School and in the Army Medical School, I had warned the members of the class that if they found in the tropics or sub-tropics an anemia they could not explain, to be sure to consider hookworm disease as a possibility.

Fortunately, Ashford who had received instruction on hookworm disease both in Georgetown Medical College and in the Army Medical School was one of the medical officers who was sent with the troops to Puerto Rico. Sternberg has more than once referred to this coincidence in conversation with me in connection with Ashford's splendid work.

Ashford (1900 a) soon brought to the attention of the American medical profession his observations on twenty cases of anemia in Puerto Rico, nineteen of which he definitely attributed to *A. duodenale.* This report was, as far as my notes show, the first instance in which hookworm dis-

ease, *confirmed microscopically as to the etiological agent,* was reported for this island, which by this time had become United States territory. The first microscopic diagnosis was made on "November 24, 1899," but the first publication was issued on April 14, 1900. At this date begins Ashford's splendid record of work on this disease,—a record of which all his friends and colleagues are justly proud.

With and shortly following the Spanish-American war a new chapter began. The essential data were:

(1) Publication (April 14, 1900 a) by Ashford (of the army) of the demonstration of hookworms in 19 cases of anemia in Puerto Rico, one of the Greater Antilles, thus confirming microscopically the interpretation of Hirsch (1883 b) and Blanchard (1888 a);

(2) Publication (May 10, 1902 v) by Stiles (then of the Department of Agriculture) that there existed in man in the United States and in Puerto Rico a new species of hookworm, *Necator americanus;*

(3) Publication (October 24, 1902 ll) by Stiles (then of the Public Health Service) and by Harris (November 15, 1902 e; of the Georgia State Board of Health) that the infection was demonstrated as widespread in our Southern States;

(4) A great increase in the literature by various authors, reporting and demonstrating cases in the South and in Puerto Rico;

(5) Extension of the campaign against hookworm disease—

a—(1903) in our Southern States by the Public Health Service;

b—(1904) in Puerto Rico by cooperation of the U. S. Army (B. K. Ashford), the U. S. Public Health Service (W. W. King), and (Gutierrez) by the Government of the island—which financed most of the work; and

c—(1909) Mr. Rockefeller's gift to finance in the South a more intensive campaign (in cooperation with the State Boards of Health and the Public Health Service) than had proved feasible (1903–1909) under the existing laws and appropriations.

ASHFORD'S DIAGNOSIS BY DEMONSTRATION OF THE PARASITE IN PUERTO RICO

As shown above, Hirsch (1883 b, 1885 a, 1888 a) and R. Blanchard (1888 a) had reported the existence of hookworm disease in Puerto Rico (apparently on basis of symptoms published in literature), but there is no evidence known to me that either of these authors had actually examined specimens from that island.

Surgeon-General Sternberg (1900: 274) reports that:

"While stationed at Ponce, P. R., Lieut. B. K. Ashford, assistant surgeon, United States Army, made a special study of the anaemia so common among the underfed natives of that island. In a number of cases he found the diseased condition to be due to the presence of the *Ankylostomum duodenale* in the intestine. His report, which is submitted below, was made the basis of a circular [No. 5] issued [1900] by

the superior board of health of the island, inviting attention of medical practitioners to the methods of recognizing and destroying the parasite."

The report in question was printed (with slight variations) in four places, namely, Ashford, 1900 a, April 14: 552–556; 1900: 274–277; 1901 a: 121–124; 1901: 121–123. Quoting from its first publication, the following passages may be noticed:

"I have the honor to report upon 20 cases of the severe anemia commonly seen among the poor of this island. * * * I was led to examine the faeces for the ova of *Ankylostoma duodenale,* and found them in great numbers. Soon after, a large dose of thymol brought away the parasites, male and female. No sooner had I stated my results to the physicians of this city than they agreed as to the diagnosis, and verified the parasite and its eggs. Their testimony is as follows: * * * Up to this time the existence of this parasite had not been proved on this island; or, if proved, not within their knowledge. * * * I was led to examine the stools carefully from the high eosinophile count, and it is certainly evident that trichinosis has a rival for high counts in ankylostomiasis. The histories of this disease have been made up to show what percentage of the people have the disease in certain countries: 25 per cent in Egypt, 20 per cent in Maitland, 52 per cent in Madras, 13 per cent in Kioto, Japan; but no percentage can be cited, as yet, for this island. Dr. B. Scheube, of Greiz, speaks in his work of its existing in the Antilles, but no island is specified, nor is the extent of the disease stated. From my own observation, and from the opinions of the resident physicians of the island, I believe it to be wide-spread and destructive. Only 20 cases have been examined, yet all save one have given me the ova of this parasite in large numbers. . . . The French, of the French Antilles, call it cachexie aqueuse and recognize its full importance; and literature is full of its ravages in South America. . . . I shall not lengthen this paper by any description of a parasite so well known and so fully described by the professor of helminthology at the Army Medical School."

Further reports are available for the West Indies as follows:

Nicholls (1900 a, May: 248). In 1897 Gulgey asserted that anchylostomiasis is endemic and prevalent all over the West Indies, "an assertion in which I entirely agree."

Nicholls (1900 b: 54–55). Anchylostomiasis in Leeward Islands, Antigua. "In these West Indian Islands."

Branch (1905: 261–262). West Indies. Found parasite in St. Kitts in 1894. "Portuguese white liver." Well known in Barbados.

DEMONSTRATION OF THE NEW HOOKWORM, *Necator americanus*

Article 21 of the International Rules of Zoological Nomenclature reads as follows:

"The author of a scientific name is that person who first publishes the name in connection with an indication, a definition, or a description, unless it is clear from the contents of the publication that some other person is responsible for said name and its indication, definition, or description."

For many years *A. duodenale* was the only hookworm known for man. But from an historical viewpoint two publications—almost universally overlooked—should be mentioned:

As pointed out by Leger (1921: 159–161), Camuset (1868: 29–30)

reports observations made by Maréchal on specimens which he considered to represent a distinct variety of *A. duodenale*. The new (but unnamed) variety differed from *A. duodenale* by three characters, namely, (1) length of body, (2) in having 14 rays in the bursa instead of 11, and (3) equatorial position of the vulva ("vers la partie moyenne").

The second difference is at first a little difficult to interpret but becomes clear if one counts the ventral rays of *A. duodenale* as single instead of double, and the dorso-median ray of the new "variety" as double instead of single.

It seems possible, therefore, in fact probable, that Maréchal recognized *N. americanus,* in spite of the fact he attributed to his new form four brownish, dorsal, hooks or teeth, instead of two ventral semilunar plates.

Lutz (1888 a: 747) (who in 1885 to 1888 was probably the most prolific author on hookworm disease in Brazil) made the interesting observation that among the worms expelled by the thymol treatment some had hooks in the mouth, but usually these hooks were absent. He concluded that "probably these [hooks] remain fastened in the [intestinal] mucosa." In all probability however the specimens without hooks in the mouth were *N. americanus*. He says:

"Meinen früheren Mittheilungen über diesen Gegenstand habe ich nur wenig beizufügen. Die daselbst beschriebene Thymolkur hat sich seither noch vielfach—und zwar auch in anderen Händen—bewährt. Eine Eigentümlichkeit derselben habe ich nachzutragen; es ist dies, dass man bei den abgetriebenen Exemplaren gewöhnlich, wenn nicht regelmässig, die gebogenen Zähne der Mundkapsel vermisst; dieselben bleiben wahrscheinlich beim Abgange des Wurmes in der Schleimhaut stecken."

Torres (1909: 19, 23, 24) and Faria & Travassos (1914: 4) refer to the foregoing observations by Lutz.

On May 1, 1902, I presented before the American Gastroenterologic Association at Washington, D. C., a paper announcing a new species of hookworm (*Uncinaria americana*) in man. The name with specific diagnosis was published (1902 v: 777–778) on May 10. At the time I was unaware of the foregoing publications by Lutz (1888) and Maréchal (1868), neither of which fulfills the requirements of Article 21 (see above).

Several authors have either intimated or stated that this new species was based upon specimens which Ashford had brought or sent to me. This would be a minor point were it not viewed in connection with certain other statements. Under the circumstances it seems wise to give the facts.

In the original publication the "type specimen" is given as No. 3310, B. A. I., U. S. Dept. of Agriculture. The catalog of the Bureau of Animal Industry shows that No. 3310 was sent in by Allen J. Smith from Texas. The Puerto Rican specimens cited in the original publication of the species were borrowed from the Army Medical Museum, as stated by Stiles in 1903 k:

"In some respects these parasites resembled *Uncinaria stenocephala* of the dog, and both Allen J. Smith and I were fully agreed that they were not identical with *Ancylostoma duodenale*. I obtained specimens from Claytor's [1901 a] case and also some material from Porto Rico which Ashford had given to the U. S. Army Medical Museum."

The first specimens Ashford furnished me were sent from Puerto Rico at my request. Dr. Maurice C. Hall has kindly given me the following record of them from the Catalogue of the Zoological Division, Bureau of Animal Industry:

"I checked on the specimens about which you asked me yesterday and find that we have 4 entries for *Uncinaria americana* collected in Puerto Rico by Bailey K. Ashford, June 11, 1902, and determined by Stiles November 25, 1902. No. 3437 appears to be the original consignment still in the collection. 3438 was returned by Stiles October 1, 1907. 3425, part of the previous lots, was sent to the Berlin Museum, and 3426, part of the previous lots, was sent to the Vienna Museum."

Ashford (1934: 45, 47, 95) states that prior to his January, 1900, visit to my laboratory he had observed that the Puerto Rican worm was without teeth. It is very difficult to harmonize this statement with earlier publications; for instance, in Circular No. 5 of the Superior Board of Health of Puerto Rico (1900), based in part upon Ashford's work, the worms are said to have 4 ventral hooks (p. 14; copied from Manson, 1898); Ashford (ibid., 27–31) does not mention the absence of hooks; I have searched in vain in Ashford's early publications for a statement on this important point. Unfortunately he apparently did not publish this observation until 1934 and the first knowledge I had of it was when I read it in his Autobiography.

The interpretation, as given by different authors, as to the practical significance of the new species is interesting as shown in the following quotations:

"With the discovery of this new parasite, the intimation just given—namely that uncinariasis has existed here for some time, but has not been recognized—may now be made as a positive statement."—Stiles, 1902 hh: 183.

"Having now an endemic species, with specimens in my possession for Washington, D. C. (patient came from Virginia), Porto Rico (Ashford's material), Cuba (specimens sent by Guiteras), and Galveston (Allen J. Smith's material), I did not hesitate to state positively in a paper published in the Eighteenth Annual Report of the Bureau of Animal Industry, and issued on September 25, 1902, that we must have in the United States an endemic uncinariasis which had been generally overlooked."—Stiles, 1903 k: 34–35.

"The discovery of a distinct American species of the hookworm is very important, as it leads to the inference that the aborigines of this country were infested with this parasite, and that the worm is probably present in all parts of the United States where the conditions are suitable for its development."—Harris, 1902 c: 776.

"The new species, interesting from a zoologic standpoint, although the mode of infection, symptomatology, morbid anatomy, treatment and prophylaxis of the disease caused by the old-world and the new-world worms are, so far as we know, precisely similar, became of tremendous importance when Dr. Stiles demonstrated for the first time that hookworm disease was crippling the South just as Dr. Ashford had

shown it to be the industrial curse of Porto Rico."—Ashford & Gutierrez Igaravidez, 1910, 1758 (5–6).

"That this worm in which I discovered no teeth turned out to be a new species (*Necator americanus*) had absolutely no practical bearing on the disease; for the new species causes precisely the same set of symptoms and is treated in precisely the same way as the *Ancylostoma duodenale*, an Old World species."—Ashford, 1934: 95.

The foregoing conclusions by Stiles and by Harris were based upon the then known geographic (i.e., only New World) distribution of the species.

An entirely new and important premise was presented by Linstow (1903 a: 526) who reported the new species in a West African chimpanzee and by Looss (1905: 430–431) who recognized this species as present in Africa in the pygmies from the Ituri Forest. This new premise justified a new deduction, namely, that since human migration had been from East to West, instead of from West to East, the presence of this parasite in America is to be interpreted as an inheritance of the slave trade. It will be recalled that about 6,000,000 negroes were transported in British ships alone, and of these about 2,000,000 died either before or shortly after the ships reached the Americas; it will be recalled also that prior to the Spanish-American war hookworm disease was reported (in part on basis of symptoms, in part on basis of the parasite) among the negroes in the Americas.

Lutz (1885 c: 2319) had already considered the slave-trade as an important factor in the distribution of hookworm disease. He says:

"In der neuen Welt hat jedenfalls der Sclavenhandel die Verbreitung des Parasiten ungemein begunstigt, wann auch vielleicht die Uebertragung nicht ihm allein zur Last fällt. Dass der Parasit in Amerika vor dessen Entdeckung einheimisch gewesen sei, erscheint mir in keiner Weise wahrscheinlich."

DEMONSTRATION THAT THE INFECTION WAS WIDE-SPREAD IN OUR SOUTHERN STATES

On October 24, Stiles (1902 11: 2433–2434), reporting on his findings during a trip in Virginia, North Carolina, and South Carolina, stated:

"All of the cases thus far examined are due to *Uncinaria americana*, demonstrating clearly that this is an endemic infection and totally independent of the cases which have been introduced from Europe, Asia, and northern Africa. * * * There is, in fact, not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part of the South, especially on farms and plantations in sandy districts, and indications are not entirely lacking that much of the trouble popularly attributed to 'dirt-eating,' 'resin-chewing,' and even some of the proverbial laziness of the poorer classes of the white population are in reality various manifestations of uncinariasis."

On November 15, H. F. Harris (1902 e: 776) reported for Georgia and Florida as follows:

"In June of the present year I made a trip to north Georgia, a region that has long been noted as one in which the inhabitants are very pale and anaemic, this con-

dition being commonly reputed to be the result of dirt eating. Here I saw many instances of what was in all probability ankylostomiasis; but as a result of the ignorance of the people and their suspicion of all strangers, a proper examination could be obtained in only four cases, in all of which the parasite was demonstrated. Subsequently a case of the disease was seen that originated in middle Alabama. During September and October I have been studying malaria in south Georgia and Florida, a region in which the people show profound anaemia even more often than in north Georgia. This condition is commonly ascribed to malaria, but my observations show that in almost all instances the sufferers have no malarial parasites in their blood, but eggs of the ankylostoma are constantly found in the feces. During my entire stay in this region I saw only one case of profound anaemia from malaria, and in this instance the patient did not exhibit the extraordinary anaemia so commonly found in those infected with the ankylostoma. I feel no hesitation in saying that time will show that by far the greater number of cases of anaemia in Georgia, Alabama, and Florida are due not to malaria but to the ankylostoma, and that this is the most common of all the serious diseases in this region. There can be no reasonable doubt that what is true as regards the States named likewise holds for the entire South. Since my first case was reported 13 other instances of the disease have been seen—11 originating in this State, and 1 each in Florida and Alabama; and if all of those encountered who were suffering from anaemia could have been examined there can be no doubt that the number would be many fold greater."

On December 18 (reprint, Sept. 25), Stiles (1902 hh), in an article written at the request of Surgeon-General Wyman, collated the cases he knew of (up to the time of the reading of proof of the article) for the United States.

In February, Stiles (1903 k (1): 37–44) gave details of his survey trip (1902) in Virginia, North Carolina, South Carolina, Georgia, and Florida, and (98–110) collated the cases known to him (up to the date of proof-reading) for the United States.

The foregoing articles were sufficient to prove the wide distribution of the disease in our Southern States, and resulted in wide-spread interest in the American medical profession.

INCREASE IN THE LITERATURE REPORTING AND DEMONSTRATING CASES IN THE SOUTH AND IN PUERTO RICO

With 1901 there was a considerable increase in the American literature on the subject. The following are a few of the papers:

1901. Allen & Behrend, 1901; Boggess, 1901 a; Claytor, 1901 a; Dyer, 1901 a; Gray, 1901 a; Guiteras, 1901 d; Hall, 1901 a; J. Am. Med. Assn. 1901, July 13; Pratt, 1901 a; Schaefer, 1901 a; Stiles, 1901 p; Yates, 1901 a.

1902. American Medicine, Phila., Apr. 19; Capps, 1902 a; Claytor, 1902 a; Greene, 1902 a; Guiteras, 1902 b, c; Harris, 1902 a, b, c, d; Hemmeter, 1902 a; Herrick, 1902 a; Kengla, 1902 a; McConathy, 1902 a; Plascencia, 1902 b; Smith, C. A., 1902 a; Stiles, 1902 gg; Stiles & Pfender, 1902 a.

1903. Ashford & King, 1903; Blumer, 1903; Bondurant, 1903; Brown, 1903, 107; Capps, 1903 a; Craig, 1903, Am. J. Med. Sc. n. 126: 798–816; Henderson, 1903 a; Julian, 1903 a; Smith, 1903, Am. J. Med. Sc. Nov.; Smith, 1903 a; Stiles, 1903, g, h, oo, uu, xx, yy; Ward, 1903 a.

1902 TO THE WORLD'S WAR

On August 16, 1902, I was transferred to the U. S. Public Health Service and immediately invited attention of Surgeon-General Wyman

to the point that while hookworm disease already existed in the United States, the return of our troops from the West Indies and the Philippines might result in the importation of additional infection; that confusion would probably occur in interpreting later events because no survey had been made in this country to trace the already existing area of infection. In order to preclude the possibility of attributing widespread infection in this country to infection imported by our returning troops, I requested orders to make a brief survey in the Southern States to establish the existing distribution. The trip was made September and October, 1902, and the preliminary report (1902 11) was published October 24, 1902. During this trip, numerous cases were found and were demonstrated to local physicians. The first discussion before a medical society was given at Macon, Georgia. At various places, with the local physicians, small clinics were held. It was during this survey that the point was established that hookworm disease in man in our Southern States is more common in the sand lands than in the clay lands, a point I had already noticed, but not published, in connection with hookworm disease in dogs and sheep. Lucas had observed that seal pups infected with hookworms were almost invariably on the sand rookeries.

In December, 1902, at Wyman's request, I presented a paper before the Pan-American Sanitary Conference at Washington, D. C., giving the results obtained in the survey. An aftermath of this address was exceedingly amusing and more important. A newspaper reporter (who has since been identified [personal letter] by Mark Sullivan as Mr. Irving C. Norwood) was present, and he reported the address with the headline that the "Germ of Laziness" had been discovered. This press story was published throughout the world, causing amusement in some circles and indignation in some quarters. My interpretation is that this newspaper reporter contributed an exceedingly valuable piece of work in disseminating knowledge concerning hookworm disease. The "Germ of Laziness" became common information. It would have taken scientific authors years of hard work to direct as much attention to this subject as Mr. Norwood did through his use of the expression "Germ of Laziness." Public health workers and the laity owe him a debt of gratitude.

Wyman, who was ahead of his time in public health matters, immediately agreed with me as to the importance of starting a campaign in the United States against this infection. As first move in this direction, he detailed me to meetings of various state medical societies. At these meetings, papers were presented on the subject, microscopic diagnosis was demonstrated, and patients were exhibited. This plan initiated the field campaign (in this country) against hookworm disease by promptly familiarizing many physicians with the new findings. In these addresses emphasis in respect to prevention was placed on two points in particular, namely, treatment of the sick and improvement of the sanitation. One

favorite expression was to the effect that the eradication of hookworm disease should be based upon "20 per cent. thymol and epsom salts [treatment] combined with 80 per cent. sanitation [prevention]."

In marked contrast to the favorable reaction of the physicians in general, a sadly amusing or amusingly sad incident once occurred, namely, a couple of backwoods physicians protested to the program committee against my preaching a doctrine which inevitably would reduce their income by resulting in a decrease in disease!

The first lectures on the subject before any medical schools south of the Potomac that I recall were at Charleston, S. C., Augusta, Georgia, and New Orleans, La. In all of these, clinics were held, using the medical students as demonstration material, for in all three institutions I recognized a number of students with symptomatic hookworm disease, and demonstrated the diagnosis microscopically; the local staffs prescribed treatment.

The first clinic in this country, as far as I can recall, to specialize for any length of time on hookworm disease was conducted at Columbia, S. C., by Dr. William Weston who had established a cotton-mill clinic about 1901, specializing on malaria. As soon [1902] as the results of the survey of 1902 became known to him, he turned his special attention in this clinic to hookworm disease, and a paper (which has been very generally overlooked) was published in 1907 in which he emphasized the importance of hookworm disease in the cotton mills and reported having treated [1902–1907] "between three and four hundred cases with practically uniform good results."

Wyman and I agreed that the second most important step was to cut off the foci of infection by improving the sanitation of the homes where infection, once introduced, spread to various members of the family, and especially to improve the sanitation at the public schools and churches where infection, once introduced, spread to different families. In harmony with this idea, a portion of Bulletin 10 (1903), Hygienic Laboratory, was given up to a discussion of sanitary privies. When the manuscript was forwarded for publication, it was returned with the request that this portion of the discussion be eliminated as it was considered that this chapter was exceedingly undignified, in fact disgusting, and had no place in a scientific article on public health. It required considerable argument to prove to the "powers that be" that this portion of the Bulletin represented the key in any plan for restricting the disease; finally the manuscript appeared in print as originally written. Following this, various articles and bulletins dealing with the subject of safe excreta-disposal were issued by the Public Health Service and by the U. S. Department of Agriculture. The latter Department distributed free 350,000 copies of a Farmer's Bulletin on this subject. Various State Boards of Health took up this phase of the problem and extended the popular dissemination

of information. Thus the second important part in a public health campaign against hookworm disease was initiated.

A number of practical points and many very amusing incidents came to light in this side of the campaign. It is to be recalled that in those days there was a great popular prejudice in rural districts against privies. The point of view was that not only were they unpleasant places but that nature's way of disposing of excreta was to expose it to the rays of the sun or to hide it in the brush. At a conservative estimate, eighty per cent of the Southern rural schools and churches did not have even a "sunshine" ("surface open in back") toilet, and the subject was not supposed to be discussed in public. On one occasion I gave a public address on this topic in a local school. Following this meeting the sheriff came to me and said that my talk had produced such public indignation that he felt it necessary to give me a bodyguard the rest of the time I was in that town! After a similar address in another state, I was urgently advised to leave town as soon as possible as it was not considered safe for me to remain there! Occurrences of this kind were all in a day's work and never worried me.

At one place there was actually a political campaign in which the question at issue was whether toilets should be built at the public school in the county seat. I was imported into the town as chief orator and after a distinctly hot campaign the "pros" won over the "antis."

During these efforts to improve sanitation, I made so many public addresses and wrote so many articles on sanitary privies that the noted chemical wit of government service (Dr. Harvey W. Wiley) gave to me the nickname of "Herr Geheimrath" (namely, "Privy Councillor").

A difficulty which soon developed was that when a family followed the advice of the health authorities and installed toilets, constipation very commonly resulted and people then reverted to "dog sanitation" as compared with "cat sanitation." This resulted in the important observation that the seats of most toilets were too high.

It was obviously advisable to finance a more intensive general campaign against soil pollution, and for this purpose extra money was necessary. An esoteric incident in this connection is that a prominent southern United States senator ("Pitchfork Tillman") became interested and offered to have a bill introduced into Congress giving the Public Health Service an initial appropriation of $25,000 for this purpose; but he made it a condition that this bill should first receive the approval of the administrative officers in the Treasury Department. Wyman took the matter up with the "powers that be," who sent strict verbal orders to me through Wyman to drop the subject. Had it not been for these orders the great cooperative movement for rural sanitation (between the U. S. Public Health Service and State Boards of Health) would have been initiated several years earlier than was the case.

The Federal Investigation (1907) into Child Labor gave me the next opportunity for field work. A study of hookworm disease in the cotton mills was continued, and this confirmed beyond question my early observation (1903) that the so-called "cotton mill anaemia" was due to hookworm disease which mill operatives and their families brought to the mills from the soil polluted farms. This resulted in the widespread interest among cotton mill managers in improving the sanitation of the cotton mill villages. It resulted also in widespread criticism from enthusiasts belonging to labor unions and child-labor organizations: complaints reached the Government: accusations were made that the cotton mills had bribed me; I was even asked how much the cotton mills had paid me and threats were made that pressure would be brought to bear to have me dismissed from Government Service. A gentleman called upon me and stated that my findings in regard to the children in the cotton mills had produced such indignation in certain quarters that he and two ladies were sent out from two different offices with instructions to disprove my statement; and that upon their return (incidentally none of the three had any medical or scientific training), he and one of the ladies reported that their findings agreed with mine but the other lady reported against my interpretations; he also stated that his report resulted in such feeling against him that he left the office to seek other employment: he stated also that the lady (a lawyer by education) who reported confirmation of my views was asked by her chief how much the cotton mills had paid her to report as she did.

At Columbia, S. C., Dr. Weston, the late Dr. Coward, and I conducted a clinic in the Olympia Mill Kindergarten School and treated a considerable number of cases. The cotton mill authorities provided a public health nurse and furnished the drugs.

One cotton mill president offered to place at my disposal $50,000 to be used in combatting the disease in cotton mills, but unfortunately, the offer could not be accepted because as a government officer I was investigating the mills, and the danger was present that this kind offer might be subject to misinterpretation from certain unfriendly quarters. Thus another chance for doing good evaporated.

A short time later a very tragic incident occurred. A friend approached me one evening in the Cosmos Club in Washington and stated that a very wealthy man wished to contribute two millions of dollars to some purpose, but he attached two conditions: first, that his name should never be made public in this connection, and, second, that he wished to do something that nobody else had ever done, thus excluding the question of libraries, colleges, and hospitals. I suggested that the gentleman finance a movement for combatting soil pollution and hookworm disease. This idea appealed to him. He promised the money and requested me to select a board of trustees and to submit a detailed plan of operation. Certain

prominent men were selected as members of this board, including Dr. William H. Welch and Surgeon-General Wyman. Arrangements seemed to be complete when I was ordered (1908) to duty with the Roosevelt Country Life Commission. The evening before we left Washington I had definite information that the gift would be announced within a week or two. Incidentally, it may be mentioned that only two persons beside myself were in the secret of the man's name. The other two persons are now dead, and I am under pledge of honor never to mention the gentleman's name in this connection.

A few days later the Country Life Commission was on a train passing through a certain state. Walter Page and I were chatting, and, as the train stopped, we purchased newspapers. One of these announced the death of the man who had promised the two millions of dollars. For several days I was almost speechless. Page thought I was ill. Finally, I told him what had occurred. He guessed the man's name, and remarked that fortunately this gentleman was not the only rich person in the country. A letter was received from my friend in the Cosmos Club stating that the man had died very suddenly, and the papers had not been signed. Thus another great opportunity for good evaporated.

This was the beginning of Page's interest in looking for an endowment for this work. Most of the details in regard to the gift by Mr. Rockefeller for hookworm disease have been published (1922) in Hendrick's Life and Letters of Walter Hines Page and (1930) in Mark Sullivan's volume 3 of Our Times.

MR. ROCKEFELLER'S GIFT

A letter from Mr. John D. Rockefeller, Sr., dated October 26, 1909, addressed to twelve gentlemen, contains the following sentence—an epoch-making sentence in southern public health matters:

"If you deem it wise to undertake this commission I shall be glad to be permitted to work with you to that end and you may call upon me from time to time for such sums as may be needed during the next five years for carrying on an aggressive campaign, up to a total of one million dollars ($1,000,000)."

It was my good fortune to be intimately connected with this health work for five years. This experience combined with my studies of southern conditions dating back a number of years before the letter in question was written, and with observations made since 1914, leads me to conclude that Mr. Rockefeller's altruistic action was as far-reaching as it was far-sighted.

Probably the most difficult problem in public health work is to put into practical application the theoretical knowledge we possess which can be utilized to reduce unnecessary death, suffering, and disease, and to increase health and happiness. Mr. Rockefeller financed a campaign of

this nature and for this purpose. The South can never adequately express its debt of gratitude to him. He it was who by his altruistic generosity financed (on the advice of that wonderful analytical mind, the late Frederick T. Gates) the hookworm campaign which lies at the basis of the general public health progress made in our Southern States during the present century, for this campaign made the South health-minded. When he attached his signature to that letter he spelled health and happiness to hundreds of thousands of persons living in the South and to millions yet unborn. It is therefore eminently fitting that in enumerating the factors operative in public health advancement in the South, John D. Rockefeller should be named as among the first.

The first definite promise from the Rockefeller organization was made to me one evening following a meeting of the Southern Board of Education which I addressed at request of Mr. Gates and when I was a guest at his summer home on Lake George. His statement was characteristically brief and to the point, namely: "The Rockefeller office will support this work."

Some weeks later I was guest at Mr. Gates's home in Montclair and far into the night we discussed details. He suggested the sum of fifty thousand dollars, and I replied that this would start the work but it would be only a drop in the bucket. He asked me to name a sum, and I suggested one million dollars. To this statement he in turn replied, "On one condition, namely, that you resign from government service and give up your entire time to direct the work." I explained to him that I thought I could present a better plan and that I would give him my answer in the morning.

Mr. Gates was enthusiastically opposed to smoking. But when I retired to my room I found there a small box of very excellent cigars. Sleep was out of the question, and by 6 A M there were very few cigars left in that box. Then I stimulated myself with a cold tub and penned an undated letter of resignation to the Public Health Service.

Mr. Gates was a great admirer of the philosophy of "Mr. Dooley," and he entertained us at breakfast by reading various essays from this author. After breakfast he and I retired to his library and he put the simple question, "What is your answer?"

I placed the letter of resignation in his hands and requested him not to mail it to Washington without giving me forty-eight hours notice by telegram. Then I unfolded to him my plan, namely:

The most important single immediate move to be emphasized was to improve the sanitation of the country schools, in order to cut off the central foci of infection. This called for close co-operation with the educational machinery and brought up the important consideration that a man widely known as a professional educator would have more influence in this line than anyone else, and, on this account, it would be best to have

the administrative officer of the work a man of that type. I suggested J. Y. Joyner, State Superintendent of Education for North Carolina, as an ideal person to fill the place. Then I suggested that Surgeon-General Wyman be requested to select officers from the U. S. Public Health Service who were citizens of the respective southern states; to offer the services of these men to the respective State Boards of Health with the understanding that the United States government would pay their salaries and the Rockefeller organization would pay all of their traveling expenses; that I give up my time, not to administration, but to public addresses before medical societies, universities, colleges, schools, and other organizations throughout the area in question; and that I still remain in government service for this purpose. The argument was that we could thus put into the field immediately personnel already trained in public health field work; further, that if I resigned from the government, the interpretation would be made that I had not been able to convince Washington of the importance of this work but had succeeded "in pulling the wool over" the eyes of the Rockefeller office.

Without committing himself, Mr. Gates agreed to take the plan under advisement, and we then discussed the personnel of a board to administer the money.

Later Mr. Rockefeller's office sent telegrams or verbal messages to the twelve men in question inviting them to meet at his office on October 26, 1909, and at this meeting it was announced that Mr. Rockefeller had decided to give a sum of money up to a million dollars to finance the work. The board organized as the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease, replied to Mr. Rockefeller's letter, selected an executive committee, talked over general plans, and adjourned. Mr. Gates and I spent the rest of the day discussing plans. He gave an announcement to the Associated Press, authorized me to send telegrams of announcement to the Surgeons-General of the United States Army, Navy, and Public Health Service and to all of the southern State Boards of Health. He also authorized me to approach Surgeon-General Wyman on the plan of detailing officers from his service to aid the work in the respective states, and he agreed not to press the question of my resigning from government service.

Upon returning to the Hygienic Laboratory the next morning, I began to receive a flood of telegrams and letters from all over the country; some were purely congratulatory, others were from persons who wished to obtain positions in connection with the proposed work. Newspaper reporters flocked in for further information and by noon Dr. Allen Freeman, of the Virginia State Board of Health, a man who is "always on the job," appeared and became a human interrogation point.

It was very illuminating to note the numerous suggestions as to how the work should be conducted and how different organizations of all types

thought that they were in a strategic position to dispense Mr. Rockefeller's money. One of the most amusing letters came from a negro preacher who suggested that he be authorized to select sick negroes and that the Board pay their expenses to a certain hospital for examination to see whether they had hookworm disease. One woman camped on my trail for about two weeks trying to convince me that an instrument she had invented was just the thing that was needed in this work and that she would explain this instrument to me for a consideration of $50,000 of the Rockefeller funds. "Patent medicine" men appeared from various quarters. One "patent medicine" firm made me an offer of a guarantee of $50,000 cash and a percentage (estimated or guesstimated at an additional $100,000) of the sales, if I would give to said firm a secret prescription to be made up and sold as "Stiles' Hookworm Cure"! My reason for not accepting the offer lay beyond the mental horizon of the representative of the firm in question.

On October 27, Surgeon-General Wyman and I held a long conference regarding the use of federal officers in a hookworm campaign. For some months past I had urged him to initiate a policy (somewhat similar to that of the Army in detailing officers to state universities and other institutions for military instruction) to offer officers of the Public Health Service as liaison officers between the Public Health Service and the various State Boards of Health, a plan since developed and now in general use.

Wyman gave his enthusiastic approval to the idea of detailing men from his service to the hookworm work. Ass't Surgeon-General Glennan, Chief of the Personnel Division, was instructed to study the personnel list and to select the men. Wyman went promptly to "the powers that be" to have the plan approved and met with a disapproval of the scheme backed by an enthusiasm greater than his own enthusiasm of approval. Had the plan been approved, the entire scheme since adopted of hearty cooperation between the State Boards of Health and the U. S. Public Health Service would have been put into immediate effect. It was a sad duty to write to Mr. Gates that Dr. Wyman had not been upheld by the administrative "powers that be."

Professor Joyner was chosen administrative secretary and I scientific secretary of the Rockefeller Commission. Professor Joyner considered the proposition very carefully and finally decided that he could not give up his work in state education in North Carolina. Walter Page was then requested to suggest another educator as administrative secretary, and the choice finally fell on Wycliffe Rose, who accepted the appointment. The plan discussed in the first meeting of the Commission was definitely adopted, i.e., to work through the medium of established official organizations (the State Boards of Health), allotting funds to each board for

increased personnel, but to await invitations from the state boards for cooperative work.

Great care was exercised in selecting the key man for each state, and these men were called together for a conference in Richmond, Virginia. The ultimate outcome was the adoption of the three methods already advocated by the Public Health Service in the period 1902–1908 as fundamental, namely, (1) discussion before medical societies to accelerate treatment in private practice, (2) extension of the free clinic system, and (3) a campaign for better sanitation. These three factors were of course basic and would immediately occur to anyone familiar with the literature, the premises, and the objects to be obtained. Statements by at least three authors to the effect that the Puerto Rican plan was taken as a basis for the organization in the United States are based on insufficient premises.

Annual conferences with the key men for the respective states were held during the campaign to compare results and to discuss improvements which might be made. Naturally, I attended all of these conferences as well as the meetings of the Commission and of its executive committee, and took part in discussions of administration. But contrary to the assumption by some persons, I did not have charge of administrative matters. This phase of the subject was in the hands of Wycliffe Rose. My part of the work consisted largely in traveling all over the South to give technical and popular addresses to professional and lay audiences and to help in clinics as occasion presented. For instance, during the first year of this work I gave 246 addresses on the subject of hookworm disease.

It was exceedingly interesting to note how certain local Chambers of Commerce, one in particular, tried to concentrate the administrative work in its locality.

Mr. Gates, who always had his telescope focused on "all corners of the earth" and whose central idea in life was to accomplish good for humanity, recommended to Mr. Rockefeller the establishment of the Rockefeller Foundation, one subdivision of which was the International Health Board. Although the work by the Rockefeller Commission ceased, this Board took over some phases of the Commission's campaign and even extended the hookworm work to foreign countries.

The cooperative work for rural sanitation, by the results of its campaign in building sanitary privies, became an important factor in inhibiting the spread of hookworm disease. The various state boards of health have carried on the work, but not so intensively for hookworm disease as when working with the Rockefeller Commission, since the state boards naturally had to combine this part of their work with other duties.

One factor in improving sanitation on the farms and thus inhibiting hookworm infection has had more influence than seems to be generally appreciated. Reference is made to the demonstration work in various

lines conducted by the U. S. Department of Agriculture. Agents of this organization have visited thousands of farms, and a number of these persons have spread the gospel of improvement in sanitation.

The sanitary work by the U. S. Public Health Service around the military camps during the World War is still another factor which should not be lost sight of, and the work of the Medical Corps of the U. S. Army in treating recruits also contributed an important element to combatting the disease.

During the World War the interest that had existed in the country schools had distinctly decreased. This was not at all unnatural, for during the life of the Rockefeller Commission special emphasis was given to the schools in this connection. Nevertheless, such improvement had occurred in school sanitation by this time that the chief centers of infection were greatly reduced in number. An outstanding example of this was presented by New Hanover County, North Carolina, which, as far as my recollection goes, was the first county in the South to complete the installation of sanitary toilets at all schools both for whites and for negroes.

An unfortunate incident occurred in 1927, namely a premature announcement was published that "it is fair to state that hookworm disease has almost disappeared from the United States." This news item was copied very widely in the lay press and, according to my observations during the years 1931–34, had the effect of still further decreasing the school interest in hookworm disease and, very unfortunately, in inducing many mothers to refuse to accept the diagnosis by physicians and consequently to decline to permit the children to be treated.

In combatting the effects of this announcement, a very quiet campaign was carried out from the Rollins College campus (Winter Park, Florida). This consisted of sending hundreds of personal letters to college professors of zoology or of biology, to county superintendents of education, and also to schools for nurses. No additional work seemed to be necessary among the members of the Southern medical profession; very few of them had taken the announcement in question seriously.

Considerable emphasis in recent literature has been placed by some authors on the influence that the improved egg-counting technique has had in reducing hookworm disease. The premises on which this conclusion is based have never been clear to me. As far as actual field work is concerned as carried on by local health officers, this technique has had the tendency to decrease the enthusiasm of these health officers, several of whom have stated to me that they did not have time or opportunity to use it, but that it was emphasized so much in literature that their enthusiasm was dampened; several health officers stated very frankly that because of the importance attached to the egg-counting method they had dropped hookworm disease from their program. The idea has been advanced that

this egg-counting method has revolutionized the hookworm campaign. Frankly admitting its value in certain laboratory investigations, I am unable to recognize any contribution of any fundamental nature that it has made to the public health field work, which is still based on the two essential points of decreasing the soil pollution and treating infected persons.

Naturally, there were a number of people who did not understand the movement, and among these were in particular four prominent editors and a very prominent clergyman. An amusing incident took place in connection with this clergyman not long after he made a rabid attack on the work, namely, I was invited to inspect for hookworm infection the children of an orphanage under his patronage! As far as I know this gentleman never publicly retracted his attack, although my information is to the effect that he admitted privately that he had committed an error of judgment in attacking the gift. Personal conferences with three of the four editors in question finally converted them to the movement, and two of these in particular made full editorial amends later for their early attacks. My personal interpretation has always been that all of these attacks did more good than harm, for they advertised the work very widely.

Not unnaturally, the work was opposed by a few outstanding exceptions among medical men. On the other hand too much can not be said of the splendid spirit exhibited by the vast majority of the medical profession of the South in support of the work.

Difference of opinion occasionally arose, but the adverse results were trivial compared with the good results accomplished. And no good purpose will be served by awakening sleeping dogs.

At the end of the five year period for which Mr. Rockefeller had given his money, the Rockefeller Sanitary Commission went out of existence.

The final obsequies were somewhat tragic. There were somewhere between 100 and 150 persons, chiefly physicians, on the pay roll, the $1,000,000 had not been exhausted, and the work had not been completed. A difference of opinion arose between Mr. Gates on the one hand and Mr. Rockefeller, Jr., and myself on the other, as to the advisability of continuing the work. As Mr. Rockefeller, Jr., expressed it; "Let us complete the job." Mr. Gates, on the other hand, felt that it was time for the Southern State Boards of Health to shoulder the full duties which theoretically belonged to them. Mr. Rose was instructed to survey the situation and to report at the next meeting of the Executive Committee. His report was presented at the next meeting as the combined recommendation of the Southern State Boards. As a result, the Commission ended its days, much to the regret expressed by Mr. Rockefeller, Jr., and myself. Later it developed that the Southern Boards had understood that the Commission had already voted to cease work and that the Boards

were called upon simply to advise as to the details of closing up the work. Accordingly, the final vote by the Commission to close the work was based upon the erroneous premise that this action was advised by the Southern State Boards of Health; and the reports of the latter were predicated upon the erroneous premise that the Commission had taken a final vote to close.

BASIC PLAN OF CAMPAIGNS AGAINST HOOKWORM DISEASE

If one reads the world's literature, published prior to 1900, one notices substantial agreement in regard to the control of the disease, namely, the two most essential and generally recognized factors are:

(1) Treatment of infected persons; and

(2) Sanitation, i.e., safe disposal of feces.

In connection with both factors there is of course more or less variation as to details, but the essential points (treatment, sanitation) are basic. For instance (to cite only a few references taken at random):

Manson (1898 a). Thymol, male fern. (548–549): Sanitation, latrine; copied almost *verbatim* in Porto Rico (1900, Circ. No. 5: 34–38).
Giles (1891 a). Sanitation.
McDonald (1899 a). Treatment, sanitation; castor oil dangerous after thymol.
Osler (1895 a: 1091). Latrines, thymol.
Scheube (1896 a). Treatment, sanitation.
Zinn (1903 e: 536). Sanitation, treatment.
Railliet (1893 a: 470). Sanitation, treatment.

The Superior Board of Health of Puerto Rico started the campaign of education of the physicians in the island by issuing (1900) its 43 page illustrated Circular No. 5 in English and Spanish. This was based on Ashford, Manson, Osler, Scheube, and Ziegler. Under treatment, thymol and male fern are mentioned. Pages 34–38 on prophylaxis are almost a verbatim copy of Manson (1898 a: 548–550) and deal with sanitation (privies), soil and water pollution.

Ashford (1900 a, 1901 a) refers to treatment, but not to the necessity of improved sanitation, although he refers to soil pollution and therefore it is to be assumed that he appreciated the point.

Stiles (1902 ll) in his preliminary report refers to the poor sanitation in explaining the severity and frequency of the infections. Later he (1903 k (1): 93–96) discusses (1) treatment, (2) sanitation, (3) disinfection by heat, dryness, cold, (4) drinking water, (5) clean hands, and (6) wearing shoes, in their relation to "prevention of hookworm disease." Still later (1904 h: 130–135) the subject was discussed in further detail.

When the Puerto Rican Commission commenced (March 6, 1904) its work (authorized by act approved February 16, 1904), it naturally adopted the working principles[2] which were already recognized, namely:

[2] See especially First, Second, and Third reports for the years 1904, 1905–06, and 1906–07; of the 1911 reprint, see especially pp. 125, 134, 157, 169, 178, 194, 211, 239.

"We are therefore left two points of attack, i.e., (1) prevention of soil pollution, (2) treatment of existing cases."—Ashford, King, Gutierrez, 1905 (1911) : 99 (134).

In its later reports,[2] the Puerto Rican Commission and its successor followed the same general lines of thought emphasizing in particular the two important points: treatment and sanitation.

As a matter of fact, the Puerto Rican Commission, beginning work in 1904, followed the lines of work recommended by the U. S. Public Health Service, 1902–1904, and the Rockefeller Commission beginning work in 1909, adopted the three principal lines which the Public Health Service followed from 1902–1908; two were identical with the two prime and fundamental factors, *treatment* and *sanitation*, and were based on the world's literature prior to 1900.

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Cf. also the statements by Kober in Sternberg's Biography, 1920: 212–213; Ireland, in Ashford, 1934, v–vi; and Ashford autobiography, 1934: 96, 98.

ECOLOGICAL STUDIES ON LARVAL TREMATODES FROM WESTERN MASSACHUSETTS

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An intensive investigation of the larval trematode fauna in western Massachusetts has shown a high percentage of mollusca infected with cercariae. One species of snail, *Pseudosuccinea columella* Say, is very abundant in this region and harbors several species of these parasites. After the life cycles of many of these cercariae had been studied, it was decided to carry out a monthly survey of the cercariae in one pond to try and determine factors that influence seasonal variations, hosts infected, and fluctuations in numbers. As pointed out by the writer (1937), a knowledge of different hosts involved and of host distribution is of utmost importance in determining such factors.

It is a pleasure to acknowledge the assistance of Mr. C. F. Holthausen and Mr. W. M. Merchant in the collection of material and study of life cycles.

REVIEW OF LITERATURE

Although much work has been done on the morphology, taxonomy, and, especially of late, the life cycles of the digenetic trematodes, a scarcity of data on the ecology of the larval stages is evident. Few detailed studies of a particular group of cercariae, infecting the same species of host, have been made. Notes on individual species are presented in many life cycle investigations, but with little attempt to correlate the ecology of the larval parasite with that of the host.

One of the first observations on seasonal distribution of cercariae was made by Manson-Bahr and Fairley (1920) who recorded for a year the occurrence of schistosome cercariae in Egypt. Soparker (1921) noted the prevalence of cercariae of *Schistosoma spindale* over a two-year period. Seasonal infection of *Nassa obsoleta* at Woods Hole, Mass., was investigated by Miller and Northup (1926). A two-year survey of the seasonal infection of *Planorbis trivolvis* Say with cercariae was carried out by McCoy (1928). Chatterji (1933) included a few notes on the ecology of an echinostome cercaria, *C. palustris*. H. M. Miller, Jr., in a series of papers (1926, 1927, 1929) investigated the behavior and reactions of several species of cercariae. The reactions of cercariae to light and mechanical stimuli were studied by Miller and Mahaffy (1930) and Miller and McCoy (1930). Diurnal variations in escape of cercariae from snails were investigated by Krull (1931), Faust and Hoffman (1934), and Crawford (1937).

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Perhaps the outstanding contribution towards the understanding of the relation of cercariae to environment is that of Wesenberg-Lund (1934). He describes many new species and discusses the biology of each group encountered. With an excellent review of literature on European cercariae, his work is an important reference. His investigations included studies on seasonal distribution, geographical distribution, multiple infections, and effect on molluscan host. E. L. Miller (1936), in a study of North American cercariae, included notes on relative abundance and distribution of many species. The effect of cercariae on the snail host was studied by Rothschild (1936).

Cort, McMullen, and Brackett (1937) carried out an ecological study for two years on the cercariae infecting a single snail species, *Stagnicola emarginata angulata* (Sowerby). Their studies, however, were made during summer months only. A careful statistical analysis was made of multiple infections found, to see if such occurrence would agree with chance expectations. In a second paper, these writers (1939) made a similar analysis of trematode infections in *Helisoma campanulatum smithii* (Baker).

MATERIALS AND METHODS

Collections of *Pseudosuccinea columella* Say were made every month for a year from a small isolated pond in South Amherst, Massachusetts. A total of 4,916 snails were examined during this period. Other molluscan species were collected also to check upon other cercariae that might be present in this pond. A study of invertebrate fauna, particularly aquatic insect larvae, was carried out to try and determine specific intermediate hosts. Tadpoles also were examined regularly for encysted flukes.

Snails were isolated in half pint milk bottles and examined periodically for escaping cercariae. After two weeks isolation, all were crushed to determine total infection. Life cycle studies included placing cercariae with various intermediate hosts and then feeding encysted stages to definitive hosts.

All cercariae were studied alive to work out body details, especially the excretory system, that cannot be discerned in preserved material, and killed either by anesthetizing in neutral red and then plunging into hot Bouin's fixative (furcocercous cercariae), or by placing under a coverslip and drawing Conant's fixative through with filter-paper (echinostome and xiphidiocercariae). These methods insured uniform fixation without distortion. All cercariae were stained with Grenacher's borax-carmine, cleared in cedarwood oil, and mounted in damar.

DESCRIPTION OF AREA STUDIED

Muskrat Hole, the pond from which snails were collected in this study, is located in South Amherst, Massachusetts. The size of the pond

varies with season. During spring it is about 50 by 75 feet, with a maximum depth in the middle of about four and a half feet. In late summer the area is reduced to about 20 by 25 feet, with a depth of only about two feet in the middle. Muskrat runs and holes are scattered all around the edges, inundated during the rainy seasons, but dry in late summer. The pond is surrounded by trees and shrubs which shade a large portion of it. Hummocks and tufts of emergent vegetation are scattered throughout. *Elodea* sp. and other species of aquatic plants grow in abundance during summer months. The bottom is covered with about 14 inches of soft muck. In winter, ice is formed to within a few inches of the bottom, often reaching a thickness of 12–15 inches. Many species of frogs, especially *Rana clamitans* Latreille and *Hyla versicolor* Le Conte, breed in this pond, and tadpoles may be found in large numbers at all times as are amphipods, insect larvae, molluscs, leeches, and spiders. A few watersnakes, *Natrix sipedon* (Linn.), have been observed. Several species of birds nest in surrounding trees. Such a pond, with a wealth of invertebrate fauna that may serve as intermediate hosts for digenetic trematodes, and a concomitant varied vertebrate fauna as definitive hosts, offers an ideal location for ecological observations on larval trematodes. *Sphaerium occidentale* Prime, *Physa gyrina* Say, and *Pseudosuccinea columella* Say occur in large numbers. *Sphaerium* is parasitized with cercariae of *Gorgoderina attenuata* Stafford, 1902; *Physa,* with *Cercaria urbanensis* Cort, 1914; and *Pseudosuccinea,* with at least six species of trematode larvae. Because of this distribution of infection, *Pseudosuccinea* was selected as the snail to be used in this investigation.

DESCRIPTION OF CERCARIAE FROM *Pseudosuccinea columella* SAY WITH NOTES ON THEIR LIFE CYCLES

(Plates I–II)

(All measurements are in millimeters of ten living specimens)

1. Cercaria of *Echinostoma revolutum* (Froelich).

This species of cercaria has been well described by various workers, especially Johnson (1920), Tsuchimochi (1924), Tubangui (1932), and Beaver (1937). Further description here, therefore, is unnecessary. It may be of interest to note that this species of fluke occurs commonly in various birds and mammals and is world wide in distribution. Muskrats in Muskrat Hole are heavily infected and mature worms have been raised experimentally in laboratory rabbits. The life cycle is as follows: eggs hatch in water and the miracidia penetrate various species of pulmonate snails. Whether or not the miracidium develops into a sporocyst or a mother redia has not been established experimentally. Studies on closely related species, however (Mathias, 1925; Rasin, 1933), would indicate that the miracidium probably develops into a mother sporocyst

which in turn, produces several generations of rediae (Fig. 1 and 2). Cercariae develop within the rediae, emerge, and may encyst (Fig. 3) in the same snail or in other snails or even in tadpoles. Worms mature in the intestine of various species of birds and mammals.

2. *Cercaria holthauseni* n. sp., a large xiphidiocercaria (Fig. 4).

Studies are still in progress on the life cycle of this species of cercaria found commonly in Muskrat Hole. Although the final host has not been ascertained definitely, evidence points to the cercaria as belonging to the sub-family RENIFERINAE.

Body length extended: 0.45–0.54, body contracted: 0.21–0.26; body width extended: 0.07–0.09, contracted: 0.15–0.20; elongate-oval in shape, concave ventrally. Oral sucker 0.06–0.07 in diameter, subterminal; acetabulum 0.06×0.07, at anterior end of posterior third of body; short prepharynx leading into thick pharynx, 0.03 in diameter; medium sized esophagus, 0.05 long, forks just anterior to acetabulum into two short ceca, 0.09 long, that extend to posterior edge of acetabulum and have thick walls with narrow lumen. Stylet (Fig. 5) 0.02×0.006, with hollow tip and posterior bulb. Tail length extended: 0.40, contracted: 0.17; tail width extended: 0.02, contracted: 0.04, usually with curled tip; no structures observed. Genital anlage consists of small ovary at postero-lateral edge of acetabulum, and two lateral horizontal testes in posterior part of body; ovary diameter, 0.10; testes diameter, 0.11. Excretory bladder Y-shaped, with constricted stem, posterior bulb and two enlarged forks; two lateral collecting tubules drain from 36 flame cells; flame cell pattern, 2 (3+3+3)+(3+3+3). Eight pairs of penetration glands, all anterior to acetabulum, empty by four ducts at anterior tip of oral sucker. Body spined all over, particularly in anterior region, diminishing posteriorly; thick spines also present in tail cavity at posterior tip of body.

C. holthauseni is more closely related to the cercaria of *Zeugorchis syntomentera* Sumwalt, 1926, described by Ingles (1933), than to other described xiphidiocercariae. *C. holthauseni* is distinguished from the cercaria of *Z. syntomentera* by its much larger size, shape of the stylet, number of penetration glands, distribution of body spines, and presence of a rudimentary ovary.

When these cercariae are placed with tadpoles, worms without their tails are soon recovered in the intestines of these hosts, stylet lost, penetration glands breaking down, genitalia enlarging. A few have been recovered also in the body cavity of the tadpoles. On feeding these infected tadpoles to newts and frogs, immature flukes resembling *Zeugorchis* have been recovered in the intestine. The life cycle, therefore, would appear to be: miracidia hatch in water, penetrate the snail and develop into mother sporocysts; these in turn produce daughter sporocysts in which cercariae develop; cercariae escape from the snail, penetrate tadpoles, and become adult in the intestine of amphibians and, possibly, reptiles when ingested by these definitive hosts.

3. *Cercaria amherstensis* n. sp., a small xiphidiocercaria (Fig. 6).

Studies on the life cycle of this species of cercaria are still in progress. Preliminary observations indicate, however, that it belongs in the family PLAGIORCHIIDAE.

Body short and broad, extended: 0.34–0.36 × 0.04–0.05; contracted: 0.15–0.17 × 0.13–0.15. Tail short, usually held close to body, extended: 0.12–0.17 × 0.03; contracted: 0.06–0.10 × 0.04–0.05. Oral sucker subterminal, 0.06 in diameter; acetabulum slightly larger than oral sucker, located just posterior to body middle, 0.06 × 0.07; very short prepharynx; pharynx small and bulbous, 0.02 in diameter; short esophagus; crura extend around edge to middle of acetabulum. Stylet hollow with posterior bulb (Fig. 7), 0.02 × 0.006. Genital anlage represented by a mass of cells at anterior edge of acetabulum and another at the posterior edge. Excretory bladder Y-shaped (Fig. 6), narrow, with medial bulge and two swollen forks; lateral tubules join forks below anterior tips; flame cell pattern typical, 2 (3+3+3) + (3+3+3). Six pairs of penetration glands at antero-lateral edge of acetabulum empty by six ducts at anterior edge of oral sucker. Body covered with spines; spines present also in tail cavity at posterior end of body. Tip of tail concave, as if enclosing an opening.

The structure of *Cercaria amherstensis* and especially the mode of life cycle indicates that this species is similar to those found in the genera belonging to the family PLAGIORCHIIDAE Lühe, 1901; emend. McMullen, 1937. A large number of cercariae have been described from this family (see McMullen, 1937: 246). As far as can be determined, *C. amherstensis* differs from these other cercariae by its short stumpy tail with a terminal invagination.

These cercariae attack tadpoles and may kill them if present in large numbers. They encyst under the skin and in the muscles (Fig. 8–9). The life cycle seems to be as follows: miracidia hatch in water, penetrate snails, and undergo development into mother sporocysts; daughter sporocysts produce large numbers of cercariae which penetrate and encyst in tadpoles. Feeding experiments indicate that amphibians and reptiles serve as definitive hosts; immature worms have been recovered from the intestines of these animals.

4. *Cercaria merchanti* n. sp., the cercaria of a lung fluke of amphibians (Fig. 10).

Body extended: 0.30–0.36 × 0.04–0.07; contracted: 0.13–0.17 × 0.14–0.17. Tail held usually close to body but may be extended to almost body length, equipped with small dorso-terminal fin; tail extended: 0.03 × 0.02, at rest: 0.10 × 0.05; tail fin width, 0.02. Oral sucker subterminal, about twice size of acetabulum, 0.06 × 0.07. Acetabulum small, at anterior edge of posterior body third, 0.03 × 0.04. Fairly short prepharynx leading to small bulbous pharynx, 0.01 in diameter; short esophagus; crura reaching beyond acetabulum to almost posterior end. Stylet (Fig. 11) narrow and elongate, with collar and hollow core, 0.04 × 0.004. Five pairs of penetration glands clustered at lateral edges of acetabulum, empty by five ducts at anterior body tip. Body without spines but covered with fine protoplasmic hairs which are easily destroyed; six papillae at anterior end. Excretory bladder Y-shaped, with posterior bulb, narrow stem, and wide forks reaching to anterior tip of acetabulum; flame cell pattern, 2 (3+3+3) + (3+3+3).

The life cycle of this species has been determined experimentally. Miracidia hatch in water, penetrate snails and develop into mother sporocysts; daughter sporocysts (Fig. 12) produce cercariae which encyst under the exoskeleton of dragonfly and damselfly nymphs (Fig. 13–14); worms develop in lungs of frogs into adult *Haematoloechus* sp. Until

further studies are completed, it is considered advisable to postpone the specific description. *C. merchanti* n. sp. resembles closely the cercariae described for lung flukes of amphibians (Van Thiel, 1930; Krull, 1931; Ingles, 1933). It differs from these species, however, by having small papillae at the anterior end and a longer and narrower stylet.

5. *Cercaria pseudoburti* Rankin, 1939, a strigeid cercaria.

A single infection of this species of cercaria, described in a previous paper (Rankin, 1939a) was found. Although attempts were made to determine the life cycle, they were unsuccessful. A knowledge of related species, however, indicates that this species penetrates and encysts in an intermediate host, probably a fish, and then becomes adult in birds or mammals.

6. A schistosome cercaria.

A single infection with a species of blood fluke was found. Attempts to determine its life history were unsuccessful. It resembles closely *Cercaria wardi* and is apparently related to that species.

ECOLOGY OF CERCARIAE

1. Behavior and Reactions of Cercariae. Each species of cercaria observed in this study has its own peculiar type of behavior. The cercaria of *Echinostoma revolutum* is a vigorous swimmer, moving by violent lashings of its tail with body tightly contracted. It swims spasmodically, with alternate active and quiet periods. At rest on the bottom, it assumes a characteristic V-shape, the body bent at a sharp angle at the base of the tail, with tail extended and trembling. As a rule, this cercaria remains close to the bottom, rarely penetrating the upper layers. On coming in contact with an object it stops, attaches its suckers, and crawls actively over the surface. If the object is an insect larva, fish, beetle, or stick, the cercaria soon drops off and resumes swimming. If it is a snail or tadpole, penetration occurs. This cercaria is, therefore, positively geotropic and thigmotropic. It tends to be negatively phototropic, since most individuals gather away from the direct source of light.

Cercaria holthauseni swims vigorously, with body held closely contracted, with alternate quiet and active periods. After swimming about near the surface, it sinks and crawls leisurely over the bottom. While falling, or when swimming, should the cercaria strike an object, it immediately becomes attached and actively crawls over it. After a short while, it sinks to the bottom. Tadpoles, feeding on the bottom, have been observed to pick up cercaria. Cercariae have been noticed also crawling in through the tadpole's respiratory opening. Active penetration into the skin was never seen. This cercaria gathers on the side of the container near the source of light and is, therefore, positively phototropic as well as positively thigmotropic.

Cercaria amherstensis is a much more vigorous and active cercaria than is *C. holthauseni*. It swims for some time, and then in a long arc drops slowly to the bottom where it rests on its dorsal surface, tail contracted and curled over the body. It crawls actively over the bottom, tail contracted but often extended and lashing. After a few minutes, it swims to the surface again and repeats the process. This cercaria is decidedly positively phototropic, aggregating on the side of the container near the source of light. It is also positively thigmotropic, crawling over any object with which it may come into contact. As soon as the cercaria strikes a tadpole, it remains attached by the anterior end only, waves back and forth, surging forward with the stylet. The tail is not used in penetration, but held tightly coiled close to the body.

C. merchanti is a very rapid and constant swimmer. The body is contracted tightly and the tail extended, whipping back and forth. The cercaria often constricts into a ball, only to unfold with a rapid extension. It is found usually near the surface at the side near the source of light. When the cercaria strikes an object it ceases swimming and crawls over the surface. If it lands on anything but an insect nymph it soon drops off. On a dragonfly nymph, however, the cercaria moves about until a soft portion between sclerites is found, especially in the neck under the cephalic chitin. That this preference is not entirely a physical phenomenon, due to the mere presence of an object, has been shown by simple experiments: Small bits of wood about the same size and shape of insect nymphs were introduced to the container and made to move at intermittent periods. Although cercariae landed and crawled about, they soon dropped off. Whether or not preference is due to a chemical stimulus has not yet been ascertained. Some reaction to shadows was also noted. A cessation of swimming was often observed when a small shadow was cast over the surface, to be followed soon by resumed activity.

Cercaria pseudoburti is negatively phototropic, exhibiting a tendency to collect near the top of the water opposite the light. Cercariae emerge primarily during the night, very few during the day. They swim constantly, tail foremost, often changing direction, in scissor-like fashion typical of furcocercous cercariae. At times, they are suspended motionless in the water, or quiet on the bottom, to resume swimming at the slightest stimulus.

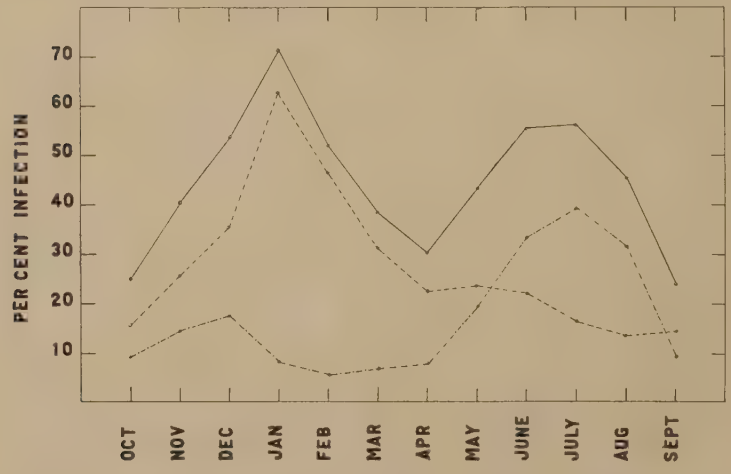
The schistosome cercaria is very active, in constant motion, swimming in typical scissor-like fashion, tail foremost. It not only swims in a weaving course, but also rotates on its own longitudinal axis. It rarely hangs suspended or crawls on the bottom. When swimming is stopped, the cercaria sinks to the bottom, rests without crawling, and then resumes activity. This larva swims close to the bottom, seldom reaching the surface. When it strikes an object the cercaria crawls about and then

drops off. Motion is stopped when a shadow is passed over it. A tendency toward a negative phototropic response has been observed.

2. Seasonal Distribution. The results reported here are for twelve consecutive months only, from October, 1937, to October, 1938. Preliminary observations, that extended over a period of one year were made in each season and showed essentially the same results that are included here. During this preliminary period, about 2000 snails were examined.

A study of the seasonal incidence of infection from month to month indicates that there are two peaks (Text-Fig. 1). About 25% of the snails are infected in October, following which there is a rapid increase in infection until a maximum of 71% is reached in January. A fairly rapid decline to about 30% in April is followed by another rise in June and July to about 55%. Towards the end of the summer and early fall, the percentage of infection again decreases to about 25%.

Observations on distribution of individual species of cercariae explains this. The cercariae of *Echinostoma revolutum* occur in but few snails (15%) in October (Text-Fig. 1). A rapid increase to about 63%



TEXT-FIG. 1. Seasonal incidence of infection of snails with cercariae. The solid line indicates percentage of total infection, the dash line with the cercaria of *E. revolutum,* and the dot-dash line with xiphidiocercariae. Note contrast in peaks in the two latter curves.

in January is followed by a fairly rapid decrease until April, following which a gradual decrease occurs throughout the summer months. The large xiphidiocercaria, *C. holthauseni,* is present in few snails (about 10%) throughout fall and winter, decreases in February to about 3% and then gradually increases to a maximum of about 14% throughout the summer. A study of the small xiphidiocercaria, *C. amherstensis,* indicates

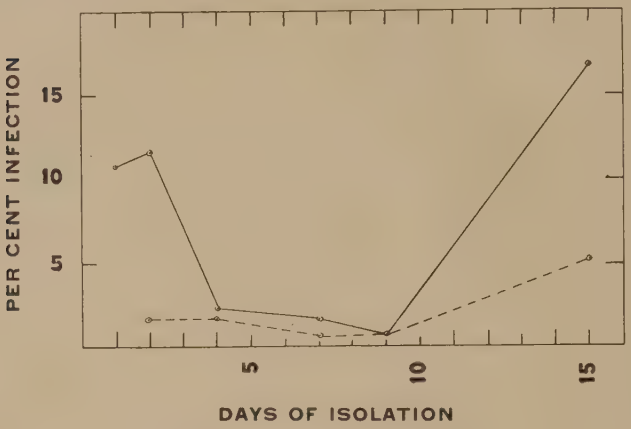
this same general trend, as does that for *C. merchanti.* The two furcocercous cercariae were found in single infections so that distribution data are not available.

An analysis of the data on seasonal incidence of total and individual infection offers an explanation for the two high peaks observed. The cercaria of *E. revolutum* has been shown to remain close to the bottom. It matures in muskrats. During spring the pond is flooded; the miracidia have less chance to infect snails and, likewise, cercariae have less chance to encyst in the snails. Percent of infection in snails, therefore, would tend to fall during early summer. In late summer but little water remains in the pond; many snails are found in the muskrat runways and may be more easily infected by miracidia which develop into rediae and cercariae during early fall. Such a condition would account for the high percent of infection during winter months. Data on total infection with cercariae that are adult in amphibians and reptiles indicate an opposite trend from that with cercariae of *E. revolutum.* The habits of the adult hosts in spring would distribute miracidia in proximity to snails. Development of these miracidia during the summer would result in elimination of cercariae during late summer and early fall. The occurrence of furcocercous cercariae in but isolated infections indicates that these worms are parasitic in hosts that come only occasionally to this pond. Such hosts are not present at all seasons, but migrate from place to place. Such infections may be termed occasional or even accidental.

A knowledge of the life cycles of the parasites involved, the behavior of the intermediate stages of the parasites, and the seasonal environmental changes, therefore, explains the presence of the two peaks in the total distribution curve. Observations on cercariae from other molluscs in the pond indicate the same general distribution as found for cercariae from *P. columella: C. urbanensis* Cort, 1914, adult in the muskrat, occurs predominantly in winter and early spring; the cercariae of *Gorgoderina attenuata* Stafford, 1902, adult in amphibians, is found primarily during summer months.

3. Shedding of Cercariae. When snails are brought into the laboratory at different periods throughout the year, it has been observed that the rate of shedding is dependent apparently on the stage of development at the time collected. As a rule, a few snails shed cercariae immediately on isolation in bottles; a sharp decrease in the number shedding is followed by a gradual increase to the maximum number of snails shedding cercariae. Text-Fig. 2 indicates such distribution and is typical for the winter months. The shedding of a few right at the start may include cercariae that were about ready to be shed or some not quite ready but which were shed due to increased temperature. A consideration of the number of miracidia penetrating the snail, and the times at which penetration occurred should also be taken into account. If but few miracidia

had penetrated the snail, just prior to its collection, the number of cercariae shed when brought into the laboratory would be negligible. On the other hand, if many miracidia had penetrated the snail, and at different intervals, extending over longer periods, then the number of cercariae shed would be considerably larger. Snails isolated in summer are usually shedding actively when collected, whereas those taken in winter exhibit the same tendency as indicated in Text-Fig. 2.



TEXT-FIG. 2. Curves illustrating trend of elimination of two species of cercariae from snails isolated in the laboratory in winter. Note sharp decrease following initial shedding, and then a gradual increase to a maximum.

4. Multiple Infections. Occasionally snails with infections of two species of cercariae were found. Hosts harboring more than two species were not observed. The usual combinations noted were: the cercaria of *E. revolutum* and *C. merchanti;* the cercaria of *E. revolutum* and *C. holthauseni.* Other combinations, including the echinostome and xiphidiocercariae, were also observed. Infection with more than one species of cercaria occurred infrequently, and when observed, in but few snails; 2.7% is the largest number found so infected at any one time. These multiple infections when present were noticed primarily during the summer months.

Along with the cercariae, chaetogasters, leeches and vorticellid protozoa have been observed. Occasionally, metacercariae of the frog bladder fluke (*Gorgoderina attenuata*) have been found encysted in the liver of the snails.

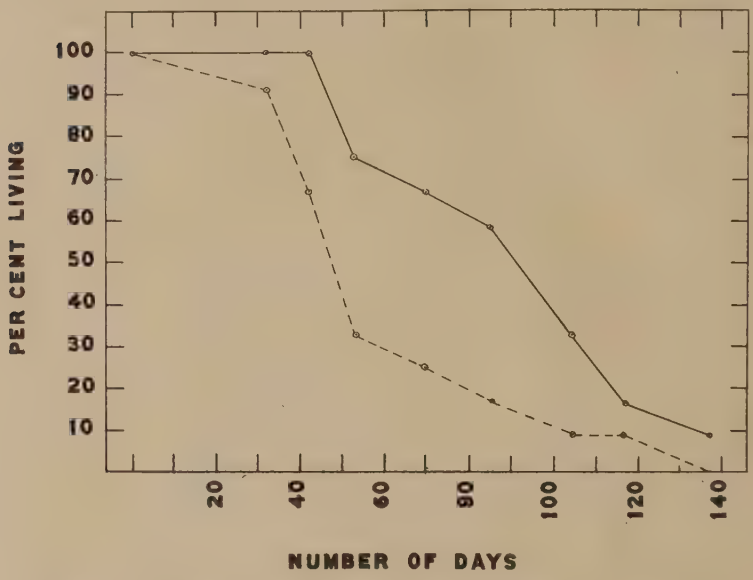
5. Development of Cercariae. Since such differential shedding periods were observed, an examination of the developmental stages of the different species were made on each collection. In February, some rediae

of *Echinostoma revolutum* were observed containing only a few mature cercariae, no daughter rediae at all. Most cercariae were immature, not even showing development of characteristic excretory granules. A few large rediae contained several daughter rediae, each of which in turn enclosed developing cercariae. It would seem, therefore, that at this period the cercaria is the important larval product. Isolation of snails in March produced many cercariae almost immediately. These were apparently prematurely shed, for they were abnormal in appearance and behavior and died in a short while. No cercaria was shed subsequently. On dissection, it was found that a few rediae contained metacercariae, other rediae contained large germ balls with little cercarial development, and no redia was found with daughter rediae. In April, a few rediae were found with mature cercariae, while many were observed to contain numerous daughter rediae. Large numbers of rediae with mature cercariae were noted in May, as well as rediae with daughter rediae. Some rediae looked as if "aborted"; that is, abnormal in shape and size, as if the germinal elements were wholly exhausted. These observations have suggested the following cycle of larval devlopment: the miracidium develops into a sporocyst which in turn produces rediae; these rediae then produce either (1) cercariae, which are potential adults, (2) daughter rediae, that produce *only* cercariae, or (3) daughter rediae, that produce cercariae *and* daughter rediae which in turn may produce the three products just listed. It seems, therefore, that the development of these latter daughter rediae may continue until a physiological limit is reached. The appearance of the so-called "abortive" rediae is believed to be this limit. Experiments to determine this and to study the germ-cell cycle of this species are in progress.

Seasonal observations on the development of the xiphidiocercariae have shown that during winter, sporocysts as a whole are filled with germ balls. Only occasionally were mature cercariae found. During summer, on the other hand, practically every sporocyst is bulging with active, mature cercariae.

6. Effect of Larval Trematodes on the Snail Host. Little or no effect on the snail host from infection with xiphidiocercariae was observed. The cercaria of *Echinostoma revolutum,* however, apparently does bring about adverse conditions within its host. Often snails have been found dying and dead, with shells gnarled and warty. Such a condition is prevalent among the large infected snails, but may be found even in smaller, half-grown hosts. Uninfected snails rarely showed these abnormalities. It was observed also that most of the infected snails that died contained large numbers of metacercariae, cercariae that had gone back into the first intermediate host and had encysted there. To test the effect of reinfection, the following experiment was carried out: 12 snails that had been isolated a month or more and had not shed any cercariae at all were used

as controls; 12 other snails that had been shedding the cercariae of *E. revolutum* for two weeks were used as infected hosts. Each snail was placed in a half-pint milk bottle with a small piece of lettuce for food; the water was changed every day. Text-Fig. 3 shows the results of this



TEXT-FIG. 3. Effect on length of life of snails in relation to re-infection with cercariae of *Echinostoma revolutum*. Solid line indicates control, or uninfected snails; broken line, infected hosts shedding cercariae that re-enter the same animal. Note decreased vitality in infected snails.

experiment. On the whole, snails that harbored hundreds of metacercariae soon died, whereas those not so infected lived considerably longer. There is a slight lag of a few days until cercariae have encysted, but shortly after this period, a rapid decline in vitality is evident. Those infected snails that lived for 100 days or more, were probably infected originally by few miracidia, and so reinfected by comparatively few metacercariae. Conversely, snails that died in a short while may have been infected by many miracidia with a subsequent heavy reinfection. This experiment shows, likewise, that snails with proper care can be kept for long periods in small containers.

7. Host Preference. The data gathered in the present study indicate that there is a considerable degree of host specificity evidenced by the various trematode larvae encountered. Other molluscs in the same pond include *Physa gyrina* and *Sphaerium occidentale.* The cercariae of *Notocotyle urbanensis* (Cort, 1914) occurs commonly in the former host.

that of *Gorgoderina attenuata* Stafford, 1902, in the latter. At no time have these cercariae been observed in *Pseudosuccinea columella,* and conversely, none of those species found in *Pseudosuccinea* was encountered in the other two species of molluscs.

The cercariae, likewise, exhibit host preference to a certain degree. The cercaria of *E. revolutum* re-encysts usually in *Pseudosuccinea.* Experiments showed that cercariae tend to penetrate uninfected snails if present, rather than to return to the originally infected host. *C. merchanti* penetrates several species of dragonfly and damselfly nymphs, and the other two xiphidiocercariae penetrate tadpoles. *C. urbanensis* encysts on blades of grass, and the cercaria of *G. attenuata* encysts in the body cavity of tadpoles (Rankin, 1939b).

DISCUSSION

In a study concerning the ecology of immature stages of an organism with a complicated life history, the complete life cycle should be understood to appreciate fully the various factors involved. This is true particularly for such animals as the parasitic worms. A knowledge of habits and distribution of the different hosts is essential in interpreting data concerning seasonal distributions, behavior, host preference, etc. Selection of a more or less isolated and small area is also advantageous. When too many species of larvae are encountered, or too wide an area is included, many important points may be obscured by an accumulation of misleading data.

A fairly definite seasonal incidence of cercariae has been observed. Total infection with cercariae tends to show two peaks, one in mid-winter, the other in late summer (Text-Fig. 1). These peaks are more clearly understood when the life cycles of the parasites involved, the habits of the various hosts, and the environmental changes are known, all of which play important rôles in such distribution. Such results are in agreement with those of many workers. Manson-Bahr and Fairley (1920) found highest percentage of infection occurring in December. Johnson (1920) suggested that the appearance of cercariae of *E. revolutum* should occur seasonally, depending on the habit of the definitive host. Although Sewell (1922) did not have a large number of snails on which to base his conclusions, he found, however, that cercariae were shed primarily in December and July. Miller and Northup (1926) found a fairly definite seasonal distribution for cercariae from *Nassa obsoleta* with peaks in December and July. On the other hand, Chatterji (1933) found that the echinostome cercaria, *C. palustris,* was prevalent in September, with a low infection in December and January. No clearcut seasonal infection was found by McCoy (1928). Various writers (MacCormick, 1923; Wesenberg-Lund, 1934; etc.) have reported no regular seasonal fluctuation in infection with larval trematodes. Such investigations may have

been on cercariae, the definitive hosts of which are more or less evenly distributed during the different seasons. Cercariae that have been observed only occasionally, such as the furcocercous forms, are larvae of parasites which infect hosts that are more or less ubiquitous. These may be considered, therefore, as only occasional or accidental parasites.

A small area tends to provide a large incidence of infection and to involve many species of parasites. This condition was noted by the writer in a previous paper (1937). Fasten (1922) observed also that restricted areas provide for rapid spread of parasitic infection and Pratt (1919) states that small enclosed bodies of water may contain more parasites than those of larger size because the fishes cannot escape by migration. On the other hand, Pearse (1924) does not consider that the size of the lake is correlated with the degree of parasitism among the fishes.

The rate of shedding cercariae is dependent largely on the extent of development at the time of collection. Ordinarily a few larvae are shed immediately on isolation. This is followed by a period of non-shedding, with a gradual increase in the definitive elimination of cercariae (Text-Fig. 2). Johnson (1920) observed that shedding would depend on time of infection of snails with miracidia. Shedding may be influenced and modified by changes in temperature, but is not dependent on such changes. Some cercariae are eliminated during the day, others at night, as has been observed by various writers (Krull, 1931); Faust and Hoffman, 1934; etc.).

Very few instances of multiple infection have been encountered. This is difficult to explain since the pond as a whole is comparatively heavily infected. The different cercariae involved in such combinations are present at the same time, especially during the summer. A much higher percentage of such infection would be expected. Sewell (1922) and Dubois (1929) have observed that multiple infections are rare. Yet Wesenberg-Lund (1934) commonly found numbers of doubly infected specimens. As Cort, McMullen, and Brackett (1937) point out, however, Wesenberg-Lund considered as double infections conditions where both metacercariae and cercariae were present in the same snail, as well as two different species of cercariae. Obviously these are two entirely different relationships. Both Sewell and Dubois reached the same conclusion concerning the mechanism that renders double infections difficult. They believe that the penetration of the first miracidium renders the host unfit physiologically for further penetration, either by destroying the chemotactic stimulus normally present or by damaging the host tissue and so preventing parasitic development. However, unless incidences of multiple infection occur in large numbers, it is difficult to determine satisfactorily whether such conditions are due to immunity or antagonism between any two species of cercariae. Cort, McMullen, and Brackett (1937) determined statistically the number of multiple infections that

would occur by chance and compared this number with the actual number found. They concluded that double infections with *Cercaria stagnicolae* and any other species occur in about the numbers that would be expected by chance; that no immunity or antagonism occurs between strigeid species; but that, on the other hand, evidence for some sort of immunity or antagonism between two of the plagiorchid cercariae was suggested. They found also, some evidence to indicate an antagonism between the echinostome and some other species of cercariae. The evidence presented in this paper does not harmonize with this latter conclusion. The cercaria of *E. revolutum* is the most prevalent parasite throughout the year and occurs sometimes in combination with other cercariae. On the other hand, some evidence for antagonism between the xiphidiocercariae was observed since such combinations rarely occurred. The mechanism for such immunity or antagonism has not yet been ascertained.

Some effect of infection on the snail host has been observed, especially with respect to the cercaria of *E. revolutum*. Old infected snails are gnarled and warty, spires broken. A flaring of the edge of the shell, forming a large shelf, has often been observed. Uninfected snails do not, as a rule, show these abnormalities. The fact that they do, however, indicates that infection with trematode larvae is not the decisive factor, but rather a contributing or modifying one. Wesenberg-Lund (1934) pointed out that infected snails are sometimes abnormally large, and noted ballooning of certain whorls, thinning and corroding of shells, and color changes. He attributed this increase in size to abnormal increase in food consumption to satisfy the demands of the parasites. Rothschild (1936) suggests that parasitism is responsible for abnormal increase in the snail, *Peringia ulvae*, but rather through destruction of gonads and other glands than through increased food consumption. According to her, variation in spire size may be due to pressure exerted by the parasite within the snail. In the present paper, although infected snails ate about twice as much food over the same period of time as uninfected individuals, it is believed that such abnormalities are influenced more by a reinfection with metacercariae and the multiplication of larval stages with the resulting destruction of body tissues, than by abnormal food consumption.

A predilection for specific hosts is evidenced to a marked degree by the various species of larvae encountered. Those found in *Physa* and *Sphaerium* have never been observed in *Pseudosuccinea*, and on the other hand, species parasitizing *Pseudosuccinea* have never been found in the other two molluscs. That some physiological difference in the molluscs brings about this specificity is evident, but just what is the mechanism has not yet been ascertained. Host preference seems to be a more or less common phenomenon and has been reported by various writers (Lühe, 1909; Mathias, 1925; Dubois, 1929; Faust, 1931; Wesenberg-Lund,

1934). Observations would indicate that miracidia are attracted to certain *species* of molluscs, whereas cercariae use *special groups* of animals as second hosts, such as insects, tadpoles, fishes, etc. This conclusion is in agreement with that of Wesenberg-Lund (1934: 202).

SUMMARY

In an ecological study of cercariae from *Pseudosuccinea columella* Say from a small pond in western Massachusetts, collections were made each month for a year. The habits, behavior, and reactions are noted of one echinostome, three xiphidiocercariae, and two furcocercous cercariae found in *Pseudosuccinea*. Life cycles of all but the furcocercous species have been tentatively completed experimentally. A fairly definite seasonal distribution of infection has been shown. Cercariae are prominent in January and late summer with low percentages of infection in between. The peak in January is due to prevalence of the cercaria of *Echinostoma revolutum* and that in summer to xiphidiocercariae. When snails are collected in winter a few cercariae are shed immediately after placing them in water; then there is a cessation of shedding, followed by a gradual increase. Temperature is a modifying, but not the determining factor. A few cases of multiple infections are reported and some degree of antagonism between species is suggested. Development of the cercaria of *E. revolutum* at different seasons is discussed. Snails infected with the cercaria of *E. revolutum* show certain shell abnormalities and a considerable reduction in vitality. As evidence of host preference, cercariae from *Physa gyrina* and *Sphaerium occidentale* do not infect *Pseudosuccinea columella*, nor do those from *Pseudosuccinea* infect the other two molluscs.

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Explanation of Plates

All drawings made with the aid of the camera lucida from living specimens; measurements are in millimeters

Plate I

Fig. 1. A third generation redia of *Echinostoma revolutum* containing a fourth generation redia.

Fig. 2. Daughter redia of *E. revolutum* filled with developing embryos. Locomotor organs, "collar," and posterior protuberance shown.

Fig. 3. Metacercaria of *E. revolutum* from *Pseudosuccinea*. Note characteristic excretory granules and oral collar of spines.

Fig. 4. *C. holthauseni* n. sp., showing flame cell pattern, genital anlage, and other body details.

Fig. 5. Stylet of *C. holthauseni*.

Fig. 6. *C. amherstensis* n. sp., showing body details.

Fig. 7. Stylet of *C. amherstensis*.

Fig. 8. Metacercaria of *C. amherstensis* in cyst taken from *Rana sylvatica* tadpole, one day infection.

Fig. 9. Same metacercaria, excysted.

Plate II

Fig. 10. *C. merchanti* n. sp., showing body details and tail fin.

Fig. 11. Stylet of *C. merchanti*.

Fig. 12. Daughter sporocysts of *C. merchanti*, with developing cercariae.

Fig. 13. Metacercaria of *C. merchanti* from dragonfly nymph, 38 days infection.

Fig. 14. Same metacercaria, excysted.

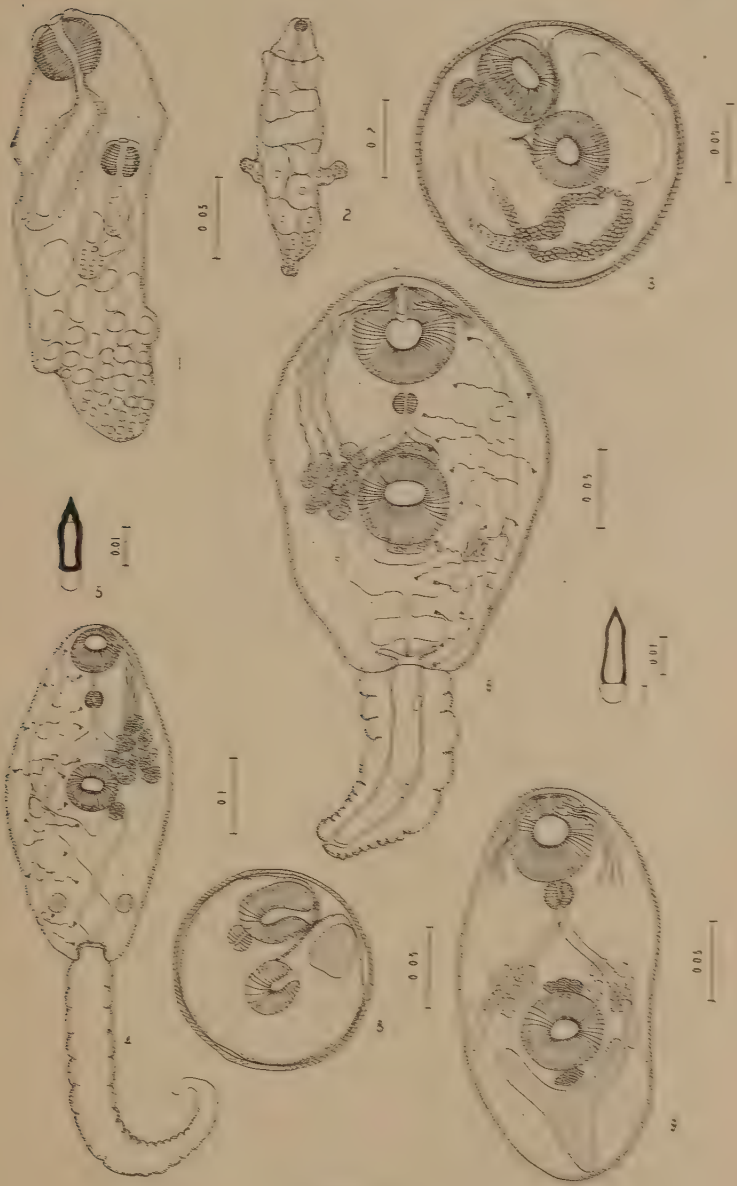

PLATE I

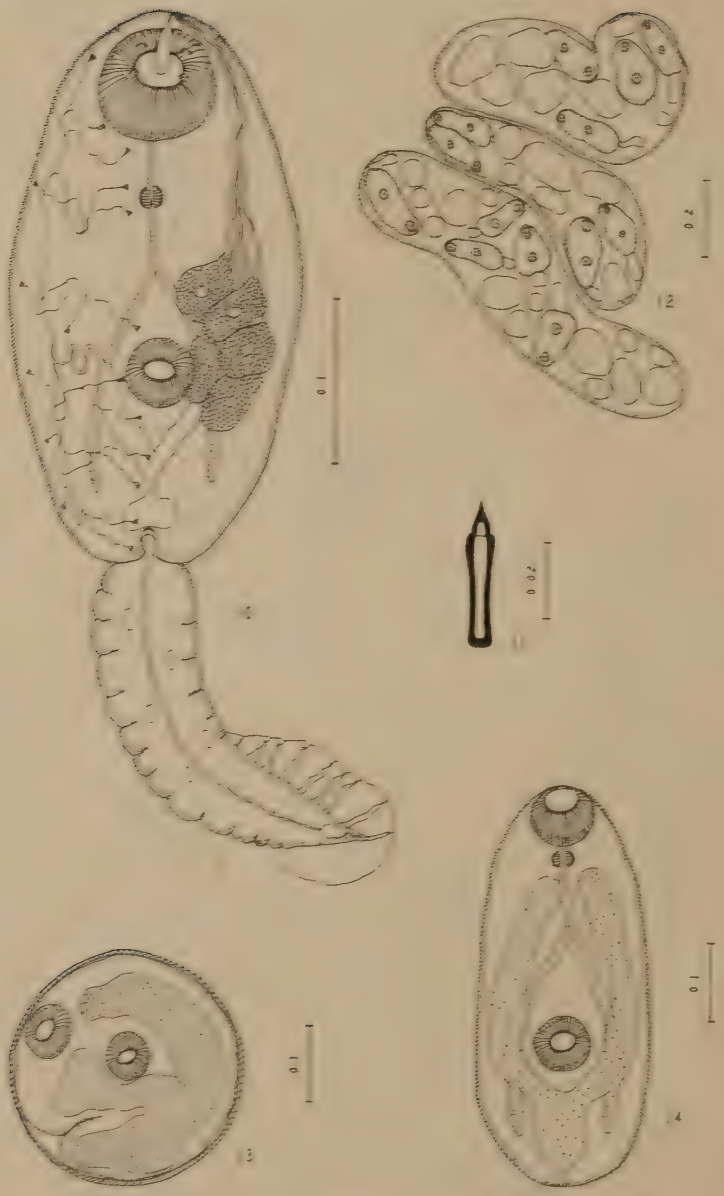

PLATE II

CHEMICAL AND MORPHOLOGICAL OBSERVATIONS UPON THE COMPOSITION OF *MACRACANTHORHYNCHUS HIRUDINACEUS* (ACANTHOCEPHALA)

THEODOR VON BRAND[1]

Data on the chemical composition of a few nematodes, cestodes and trematodes have been published by several investigators, but so far practically nothing is known about the biochemistry of acanthocephala. Weinland and Rudolph (1910) performed one glycogen analysis on freshly taken *Macracanthorhynchus*. Schwartz (1920) published a note on enzymes and hemotoxins in their body fluid and Mueller (1928 29) reported a nitrogen and sulfur analysis of the cuticle of the same worm. The lack of more complete information concerning the physiology of this group is regrettable since these worms are highly specialized in their body structure and show a remarkable adaptation to parasitic life. The following investigation was undertaken in order to secure some information concerning the composition of one of the most readily available of these forms, ***Macracanthorhynchus hirudinaceus***.

MATERIAL

All the worms used in this investigation were freshly collected and used a few hours after the slaughter of the pigs. I am indebted to the packing companies of Swift and Co. and Wilson and Co., Chicago, for an ample supply of material. The helminths were received in most cases with the pieces of intestine to which they were attached. After freeing them, they were washed with physiological saline, wiped dry with filter paper, weighed and immediately used for the determinations. The worms at room temperature were quite motionless, but began to move if brought into normal saline of 37° C. On the whole, however, they were quite sluggish.

Females were used almost exclusively for the chemical work. It was much easier to obtain a sufficient amount of material from them than from the smaller males. The latter, however, were used for some of the morphological observations.

GENERAL CHEMICAL COMPOSITION

The dry substance was determined by drying worms at 100° C until the weight remained constant. The inorganic substances and the nitro-

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[1] A contribution from the Biology Department, Barat College of the Sacred Heart, Lake Forest, Ill.

gen[2] were determined on aliquots of the dry substances secured during these determinations, the former through incineration of dry substance, the latter according to Kjeldahl's method. Pflueger's method was used for the determination of the glycogen, and Kumagava and Suto's procedure for that of the ether extract.

The results of these determinations are summarized in Table 1. While the figures recorded need little comment, two points should be emphasized.

TABLE 1.—*General chemical composition of females of* Macracanthorhynchus hirudinaceus

	Mean value and (extremes)	Total number of worms	Number of determinations
Weight of one living female in gm.	5.7 (3.8–8.3)	159	19
Dry substance in per cent of fresh substance	11.5 (8.7–15.0)	30	6
Inorganic substances in per cent of fresh substance	0.58 (0.45–0.73)	30	6
Nitrogen in per cent of fresh substance	1.28 (1.05–1.41)	30	6
Protein (= N × 6.25) in per cent of fresh substance	8.0 (6.6–8.8)		
Glycogen in per cent of fresh substance	1.13 (0.98–1.27)	79	8
Ether extract in per cent of fresh substance	0.95 (0.90–1.17)	50	5

Glycogen and fat content vary only slightly from determination to determination. Especially for the former substance, the variations are much smaller than in other helminths. This would seem to indicate that these worms might be especially suitable for experiments on metabolism, but preliminary experiments showed that they are difficult to keep alive in vitro for longer periods. The other fact worth noting is that the glycogen content is relatively low. As a matter of fact, it seems to be the lowest so far found in any intestinal helminth. The low glycogen content is the more curious, as Weinland and Rudolph (1910) found in the same worms 3.86% of glycogen. The average weight of their worms was, however, only 0.7 gm, indicating that they probably used males. A single determination which I was able to perform on males yielded a value of 2.14% glycogen. It seems possible that a marked sexual difference in glycogen content occurs, contrary to what has been found in *Ascaris* (v. Brand, 1937).

In order to get information about the importance of certain organs as storage places, some experiments were carried out in which the material had been separated as follows: 1. Hypodermis + muscles. This part contained in addition the cuticle, nervous system, excretory system and those parts of the reproductive system fixed by muscles to the body wall. 2.

[2] The nitrogen determinations were performed at the Department of Parasitology, University of Chicago. I am indebted to Dr. W. H. Taliaferro for allowing the use of the facilities of his laboratory and to Dr. D. H. Campbell for help in assembling the apparatus.

Ovaries and eggs, separated from the body fluid by centrifugation. 3. Body fluid, secured by incising the anterior part of the body and squeezing it out quantitatively by gentle stroking. The combined body fluids of all the worms used in one experiment were collected and centrifuged in order to get a separation from the sexual cells. Table 2 shows the

TABLE 2.—*Distribution of substances in certain organs of* Macracanthorhynchus hirudinaceus *females. Mean values (and extremes)*

		Hypodermis + muscles	Ovaries + eggs	Body fluid
Fresh weight in per cent of total weight		46 (40–52)	12 (8–16)	42 (36–52)
Dry substance	per cent of fresh substance	16.8 (16.0–17.5)	23.4 (21.9–24.8)	3.7 (3.5–3.9)
	per cent of total dry substance	61 (56–66)	27 (22–33)	12 (11–12)
Ether extract	per cent of fresh substance	1.3 (1.2–1.3)	1.9 (1.8–2.0)	0.2 (0.21–0.22)
	per cent of total ether extract	61 (56–66)	30 (25–35)	9 (9–9)
Glycogen	per cent of fresh substance	1.5 (1.4–1.5)	0.9 (0.5–1.2)	0.2 (0.09–0.28)
	per cent of total glycogen	80 (71–89)	12 (7–17)	8 (4–12)

The figures for the fresh weight are based on 4 experiments with a total of 71 worms weighing 305 gm.
The figures for the dry substance and the ether extract are based on 2 experiments with a total of 35 worms weighing 148 gm.
The figures for the glycogen are based on 2 experiments with a total of 36 worms weighing 157 gm.

results of these experiments. The body fluid was present in very large amounts[3] and this explains the low content of dry substance found in determinations on whole worms. The highest percentage on dry substance was found in the reproductive cells. The largest parts of the polysaccharide and the ether extract were deposited in the skin and muscles and this was more pronounced in the case of the former substance. The reproductive cells contained a distinctly higher fraction of the total ether extract than of the total glycogen.

CHEMICAL OBSERVATIONS ON SOME BODY CONSTITUENTS

Inorganic substances. No quantitative analysis concerning the composition of the inorganic substances was performed, but some qualitative tests were carried out. The presence of the following substances was ascertained:

Cations: Na, K, Mg, Ca, Fe.

Anions: PO_4, SO_4, CO_3, Cl.

The presence of none of these substances needs special comment, since they all are widely distributed in living organisms.

[3] The amount of body fluid seems somewhat high. It is known that acanthocephala imbibe fluids rapidly if placed in hypotonic media. The handling of the pig intestines in the packing companies was beyond my control, and perhaps they had been in contact with water.

Polysaccharide. The polysaccharide isolated according to Pflueger's method shows the characteristics of glycogen. It is easily water soluble, the aqueous solution is opalescent and gives a dark red-brown color with iodine. It does not reduce Fehling's solution, but after heating with 2 per cent HCl or after standing for some time with filtered saliva, it reduces strongly. The sugar formed after hydrolysis with HCl is glucose, as evidenced by the fact that the melting point (204° C) of the osazone is identical with that of glucosazone.

There is, however, some possibility that this polysaccharide contains, besides a large part of glycogen, a small part of another polysaccharide. One gram of polysaccharide was digested for 24 hours with filtered saliva at 37° C, then one alkaline and one acid precipitation with alcohol were performed. The precipitate was dissolved in water and heated for one hour with KOH on a boiling water bath, after which it was precipitated with alcohol. After drying, the precipitate was dissolved in 5 ml of distilled water, neutralized with acetic acid and again digested for 24 hours with saliva. Despite this digestion, no reduction of Fehling's solution could be obtained. The solution was again heated with KOH and a final precipitation with alcohol was performed. The small precipitate was dissolved in 2 ml of distilled water. This solution was only very slightly opalescent. It was ascertained that one-half gave no color reaction with iodine and did not reduce Fehling's solution. The other half was brought to an HCl concentration of 2 per cent and heated for an hour on a boiling water bath. It then reduced Fehling's solution markedly.

Obviously then, a small amount of polysaccharide was present which escaped the digestion with saliva. Further experiments with a much greater amount of material are necessary before its nature can be determined. It may, however, be pointed out that the fact of its escaping salivary digestion and giving no color reaction with iodine would seem to point towards galactogen. On the other hand, no precipitate was found upon heating 2 ml of a 5 per cent solution of original polysaccharide with alkaline copper solution, though according to May (1934) under these conditions a precipitate of galactogen copper can be observed, if at least 0.25 mgm of galactogen are present. The possibility that the substance in question is only an intermediate substance formed during the digestion of glycogen, is not entirely ruled out. It is, however, not a very likely explanation, since a procedure quite similar to that used above permits the quantitative separation of galactogen from glycogen in *Helix*.

Ether extract. About 40 gm of pulverized dry substance was extracted for several hours in a Soxhlet apparatus with ether, whereby 3.06 gm ether extract were secured. It was redissolved in a very small amount of ether and precipitated with acetone. The acetone soluble and insoluble fractions were separated by filtration.

The acetone insoluble fraction weighed 0.82 gm. It was dark brown and solid at room temperature. In this fraction a large amount of phosphorus occurred, as demonstrated in the usual way by precipitation with ammonium molybdate. It consisted, therefore, of phospholipids (phosphatides, lecithin).

The acetone soluble fraction was semi-fluid at room temperature, and weighed 2.24 gm. It was dissolved in 96 per cent alcohol and heated for one and a half hours with strong alcoholic KOH solution on a water bath (reflux condenser). The alcohol was then evaporated and the product dissolved in water.

By extraction with ether in a separatory funnel, the unsaponifiable matter was obtained. It was represented by a mass of slightly yellowish crystals weighing 0.74 gm. After purification with Norit A and slow crystallization from ether, colorless crystals forming clusters of needles appeared. The color reaction of Salkowsky and that of Liebermann-Burchard were identical with those given by cholesterol. The melting point of the isolated substance was 115° C and, after two further recrystallizations from 96 per cent alcohol, 128° C. This is considerably lower than the melting point of cholesterol (148° C). Further investigations will have to decide whether the unsaponifiable matter of *Macracanthorhynchus* represents a mixture of cholesterol and some other higher alcohol, or whether it is another sterol. It is known that in some invertebrates sterols different from cholesterol are found.

The free fatty acids were secured by ether-extraction after acidulating the soap solution in the separatory funnel. This fraction weighed 1.35 gm. It was light brown and fluid at room temperature. It was dissolved in 96 per cent alcohol, neutralized with KOH and precipitated with hot lead acetate solution. The resulting lead soaps were washed repeatedly with hot water, then dried and dissolved in hot benzene. After cooling to 10° C, a precipitate formed which was separated from the solution by filtration.

This precipitate yielded, after acidification and extraction with ether, 0.06 gm of fatty acids, a fraction representing the saturated fatty acids. Fifty mgm were dissolved in 5 ml alcohol and titrated with the help of a microburette with N 10 alcoholic KOH, using phenolphthalein as the indicator. They required 1.64 ml N 10 KOH. The molecular weight was therefore 274. This is an intermediate figure between stearic and palmitic acid and it is quite likely that a mixture of these two acids was present. The melting point was 50° C and therefore considerably lower than should be expected. This, of course, is due to the fact that the separation of saturated and unsaturated fatty acids over their lead salts is never immediately quantitative. Owing to the scarcity of the material a further purification was impossible.

The unsaturated fatty acids, recovered from the filtrate of the benzene solution, weighed 0.98 gm. This fraction was slightly yellow and fluid at room temperature. For neutralization of 115 mgm 3.95 ml N 10 alcoholic KOH solution were required. The molecular weight was therefore 292. The difference from oleic acid (molecular weight 282) is small and probably within the limits of error of the titration procedure used. Unfortunately, the remainder of this fraction was accidentally lost before the iodine number was determined.

The fluid which had remained in the separatory funnel after extraction of the unsaponifiable matter and the fatty acids, was made slightly alkaline with K_2CO_3. Then the greatest part of the fluid was evaporated

at a temperature not exceeding 50° C. The resulting syrup was thoroughly mixed with water-free sodium sulphate and extracted with acetone in a Soxhlet apparatus. In this way a slightly yellowish, viscous fluid, weighing 0.06 gm was secured. It gave upon heating with potassium bisulphate a typical acrolein reaction and therefore was doubtless glycerol.

In per cent of total ether extract the composition of the fat is as follows:

Phospholipids	27
Unsaponifiable matter	24
Saturated fatty acids	2
Unsaturated fatty acids	32
Glycerol	2
Unidentified lipoids	13

It should be noted that the unsaponifiable matter represents a large part of the total ether-extract. This corresponds to the findings in other helminths. Similar findings have been reported in *Ascaris* (Flury, 1910), *Fasciola* (v. Brand, 1928) and *Moniezia* (v. Brand, 1933).

MORPHOLOGICAL OBSERVATIONS

Glycogen. Two methods are possible for a study of the distribution of the glycogen in the body, the chemical determination and differential staining. The former gives quantitative results, but no exact information about the localization in the tissues, since, in general, organs but not the single tissues can be separated. The results of these determinations have been shown in a preceding paragraph. Differential staining is not a quantitative method, but it gives more details about the deposition of the polysaccharide. The method used was the staining of sections of the worms (fixed with Carnoy's solution) with concentrated Delafield's solution and Best's carmin. Controls were carried out by staining corresponding sections in the same way after having digested them for one hour at 37° C with filtered saliva. Another control consisted in staining sections with Lugol's solution. Similar studies have been carried out previously with nematodes (v. Kemnitz, 1912), trematodes and cestodes (Ortner-Schoenbach, 1913), but never with acanthocephala. The only recorded observation seems to be Meyer's casual remark that he found glycogen in the lacunar system of the hypodermis of *Neoechinorhynchus rutili.*

Large amounts of glycogen were found in the subcuticula (Fig. 1). It was localized in its deeper layers and occurred in small granules along the fibers. The lemnisci, however, were glycogen-poor, indeed sometimes they seemed to be totally free of glycogen. The occurrence of glycogen in the hypodermis is interesting, since it demonstrates that this tissue is not only an organ of resorption—obviously the polysaccharide cannot have been resorbed as such,—but that it is one of the most important storage places of the body. It seems likely, furthermore, that the glycogen

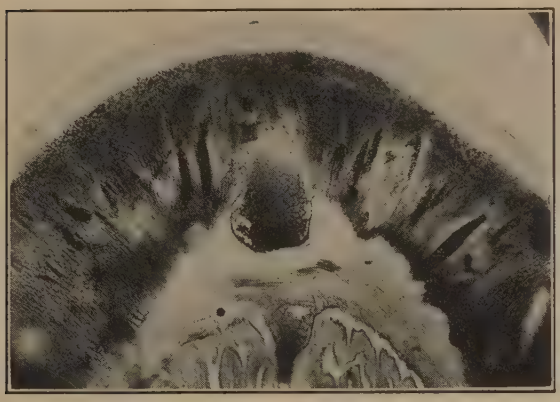
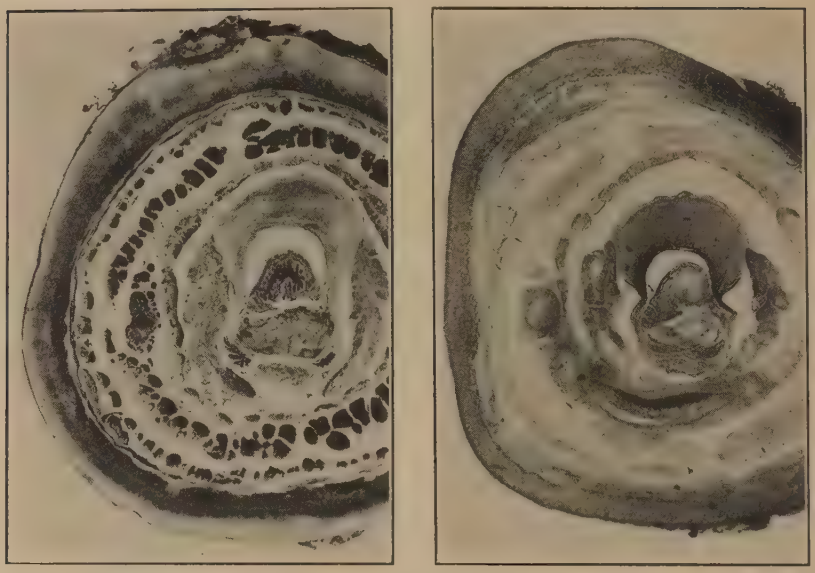


FIG. 1. Section through the body wall of *Macracanthorhynchus* female. Fixation: Carnoy's solution. Stain: Best's carmin, no counterstain. ×107. Accumulation of glycogen in subcuticula and lacunae. In saliva-digested control sections the whole section appears as light as do the muscle layers here.

has been synthesized in the subcuticula, perhaps from substances resorbed through it (sugar?) from the intestine of the host. Large amounts of glycogen occur also in the lacunar system of the subcuticula (Fig. 1.). Both large and small lacunae are often full of glycogen granulations. The nuclei lying in them are bathed in a glycogen-rich fluid, but are themselves glycogen-free. Obviously glycogen can be transported through the lacunae from one part of the body to the other.

It is rather remarkable that glycogen is transported as such, since in most cases, physiologically comparable, it is first broken down. This is probably because its very large molecule could not easily go through cellular membranes. The explanation must be sought in the peculiar structure of the subcuticula of the acanthocephala. The lacunae are essentially slits between the fibers of the hypodermis and are not lined by cellular walls. A glycogen solution will therefore have no difficulty in moving freely from the subcuticular tissue to the lacunae and perhaps *vice versa.*

Equally important as glycogen reservoirs are the muscles (Figs. 2a and 2b). Glycogen is found mainly in the protoplasmic part. Its deposition in the longitudinal and circular muscles of the body wall can be compared directly to the glycogen accumulations found in the plasma bulbs of the corresponding muscles in nematodes. One difference, however, is that in nematodes the glycogen occurs frequently in large uniformly stained patches, whereas in *Macracanthorhynchus* it is, as a rule, found in the form of granules or filaments. This difference may be due to a difference in concentration. It is probable that in the living muscle the glycogen is dissolved in the cell fluid and that it is only precipitated by



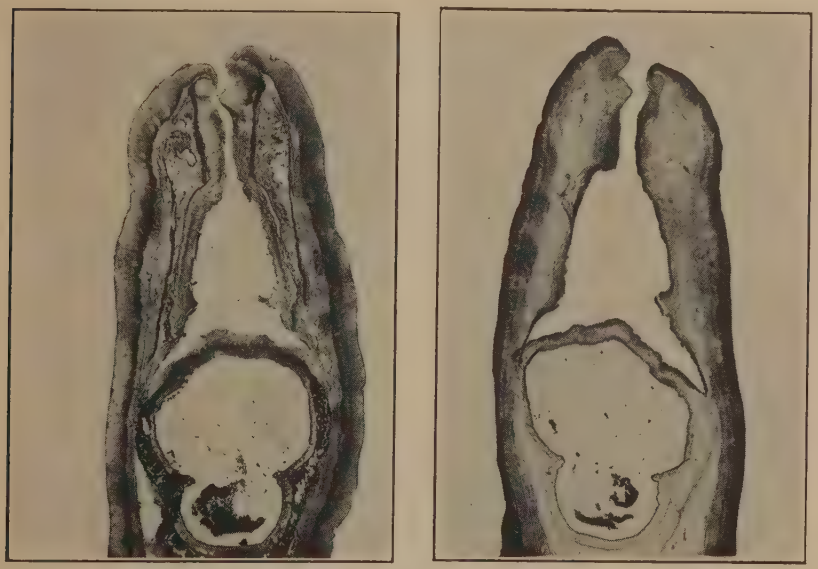
FIGS. 2a and 2b. Cross section through *Macracanthorhynchus* female in region of proboscis ganglion. Fixation: Carnoy's solution. Stain: Best's carmin, no counterstain. 2a stained directly, 2b after one hour digestion with saliva. ×50. Marked glycogen content of the muscles, as evidenced by the contrast offered by the two pictures.

the fixative. The amount of muscle glycogen seems to be higher in the anterior and posterior region of the body, whereas the middle region seems in many cases to be glycogen-poor.

The glycogen deposition is similar in most of the muscles lying in the body cavity, for example the muscles attached to the receptaculum, or to the reproductive organs, or the muscular parts of these. Indeed they seem to store rather more glycogen than the muscles of the body wall. Frequently, though not regularly, the glycogen is not only confined to the plasmatic parts of the muscles, but is also found covering the contractile fibers. This again is much more pronounced in the muscles lying in the interior of the body cavity.

Two muscular structures need special mention. The inner wall of the receptaculum is represented in its apical part by a sac filled with a fluid substance, corresponding to very fluid protoplasma (Meyer 1932/33). This part is extremely rich in glycogen and stains deeply with iodine or Best's carmin. The distal part consists of circular muscle lamellae crossed by connective tissue. It is more glycogen-poor, the glycogen occurring here in the form of varying sized droplets between the lamellae.

The muscular wall of the bursa copulatrix of the male is thick, but the muscle has a much looser texture than the body muscles. It is extremely rich in glycogen (Figs. 3a and 3b) and it should be emphasized that the



FIGS. 3a and 3b. Longitudinal section through posterior end of *Macracanthorhynchus* male. Fixation: Carnoy's solution. Stain: Best's carmin, no counterstain. 3a stained directly, 3b after one hour digestion with saliva. ×50. Great accumulation of glycogen in the muscular part of the bursa copulatrix as evidenced by the contrast offered by the two pictures.

glycogen occurring here is extremely water soluble. Its full amount can only be demonstrated in sections stained directly with Best's carmin. In sections stained previously with hematoxylin much glycogen is always lost, whereas in other regions of the body no such danger exists.

It is interesting to note that glycogen occurs in the nervous system. It is found in the form of fine granules in all the cells contained in the proboscis ganglion and, in the male, the genital ganglion. None of the different cell types described by Kochanowski (1924) seem to be especially favored. This seems to be the first case of glycogen in the nervous system of a parasitic worm. It has been reported as glycogen-free in *Ascaris* (v. Kemnitz 1912), trematodes and cestodes (Ortner-Schoenbach 1913). In free-living worms its occurrence seems to be variable. Several forms have been investigated by Gelei (1909/12), Erhard (1911) and Vialli (1927).

The excretory system of worms was in general, glycogen-free, but in

some instances the plasma of the flame cells and the walls of the excretory tubes gave a marked glycogen reaction. The only other occurrence of glycogen in excretory organs of worms has been described by Vialli (1927) for the leech *Haemopis*.

The body fluid in stained slides showed some glycogen. It was apparently precipitated around the coagulated proteins of the body fluid. This follows from the observation that in sections stained with hematoxylin and Best's carmin this material appeared red or pink, whereas in slides digested previously with saliva it was blue, without, however, being much reduced in size. Judging from the intensity of the reaction, it would seem likely that the body fluid of males contains a larger amount of glycogen than that of females.

It has already been mentioned that all the muscular parts of the reproductive tracts of both sexes are rich in glycogen. It remains to consider the non-muscular parts. The testes were always glycogen-free. The cement glands, however, regularly showed some glycogen, and often the amount of polysaccharide was quite large. It was found in the form of granules between the granular and filamentous masses of secretion which stain deep blue with hematoxylin. The significance of the presence of carbohydrate at this place may lie in the fact that some secretions of protein nature, the mucins for example, contain relatively large amounts of carbohydrate. It is conceivable that the glycogen is the mother substance for such carbohydrate, though the secretion itself seems not to consist of mucin proper. The glycogen nature of the granulations in question has been ascertained further both by their digestibility with saliva and their typical color reaction with iodine. A confusion with mucin, known to react positively with Best's carmin, is therefore ruled out.

The ovaries contain a varying amount of glycogen, which is confined to the stroma. The developing eggs in the ovaries and the early cleavage stages show no glycogen. In the older eggs, however, regularly much polysaccharide is found. It is as a rule localized in the center of the larva, in the region of the "Embryonalkern" and forms generally one large patch of more or less irregular outline (Fig. 4). Sometimes several smaller polysaccharide masses are present. Intermediate stages between eggs without and with much polysaccharide are but rarely seen; it seems to appear rapidly.

A fact requiring special discussion is the observation that the polysaccharide contained in the eggs is, in the histological slides, difficult to digest with saliva. Even after two hours digestion at 37° C relatively large masses still can be stained with Best's carmin. The carbohydrate must either be associated in the eggs with some other substance protecting it to a certain degree from salivary digestion, or it is glycogen mixed with another polysaccharide resistant to saliva. In a preceding paragraph the possibility that such a substance occurs in the worm body has been dis-

cussed. That at least a large part of the substance stained by Best's carmin is carbohydrate and that it is not a pseudoreaction due to some other substance is demonstrated beyond doubt by the relatively large amount of polysaccharide which can be isolated from the sexual cells by Pflueger's method.

Fat. The morphological features of the fat deposition were primarily studied on sections of worms fixed with 1 per cent osmic acid and embedded in paraffin. Admittedly some fat may have been lost during the embedding process, since it is well known that fat treated with osmic

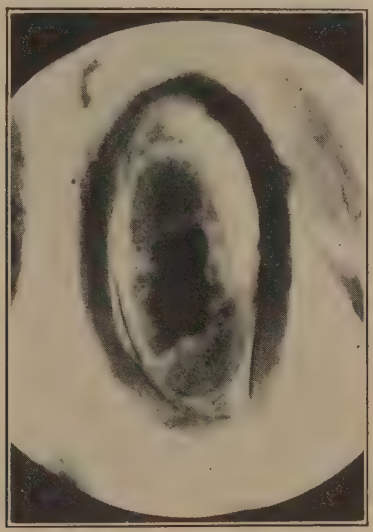


FIG. 4. Section through ripe egg of *Macracanthorhynchus hirudinaceus*. Fixation: Carnoy's solution. Stain: Delafield's hematoxylin—Best's carmin. ×700. Large patch of glycogen in center of embryo.

acid does not become totally insoluble in benzene or chloroform, both of which were used as clearing agents. Sudan III was used as a control on ovaries, eggs and some other easily isolated parts, but owing to a lack of suitable equipment no frozen sections could be stained with it. It should always be kept in mind in investigations of this type that any staining method will show up only a part of the substances representing the fat determined by chemical methods.

Like glycogen, fat is deposited in relatively large amounts in the deeper regions of the hypodermis (Fig. 5), where it occurs in the form of varying sized droplets between the fibers. The amount of fat demonstrable by osmic acid in the subcuticula seems not to vary much from indi-

vidual to individual or between worms of the two sexes. In the region of the head and, in the female, on the most posterior parts of the body, definitely less fat was found than in the much larger middle region. The lemnisci were regularly fat-free. The lacunar system of the subcuticula was found to contain no fat except an occasional small droplet. This is a marked contrast to many other acanthocephala, where frequently the lacunar system has been found to be filled with droplets of fat or oil (Saefftigen 1885, Hamann 1891, and Meyer 1932/33).

FIG. 5. Cross section through female of *Macracanthorhynchus hirudinaceus*, middle region. Fixation: 1 per cent osmic acid. No further stain. ×100. Fat droplets in subcuticula and great accumulation of fat in ovary.

The muscles were in general fat-free, though sometimes a few droplets were seen in the protoplasmic parts. In one instance a marked fat content of the musculus invaginator proboscidis was noticed.

In body fluid, nervous system, ligament and excretory system no fat could be demonstrated with the morphological methods used. Special attention was paid to the question whether fat droplets are to be found in the lumen of the excretory tubes, since such an excretion of fat has previously been described in *Fasciola* (v. Brand and Weinland 1924, Vogel and v. Brand 1933) and, to a lesser extent in *Moniezia* (v. Brand 1933). However, nothing of this kind was seen in *Macracanthorhynchus*.

The reproductive systems of both sexes contain some fat. In the

male it was found chiefly in the modified hypodermis covering the bursa copulatrix, whereas its muscle layer contained but little fat. The cement glands were fat poor and also the testes showed only a few drops of fat.

In the female a considerable amount of fat was found in the vagina (Fig. 6) and in the inner layer of the uterus. The exact localization and amount of morphologically demonstrable fat here is difficult to determine with osmic acid, since definite signs of fat loss by solution were noticed. The ovaries contained a very large amount of fat (Fig. 5); the droplets

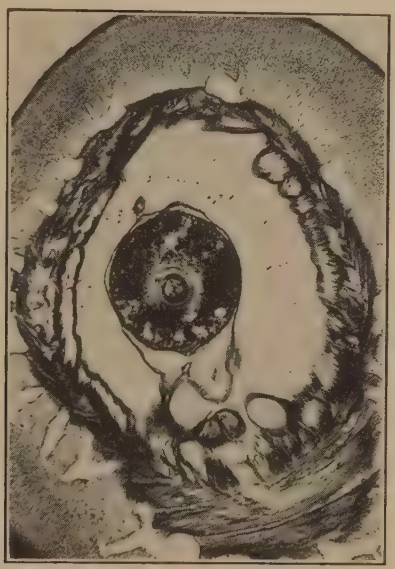


FIG. 6. Cross section through female of *Macracanthorhynchus hirudinaceus*, posterior region. Fixation: 1 per cent osmic acid. No further stain. ×100. Many fat drops in wall of vagina. Egg passing through vagina has inner wall deeply blackened. No noticeable fat content of subcuticula.

were closely packed in the stroma. This fact, and the occurrence of a few fat droplets in the young eggs has been noticed by other workers (Meyer 1932/33). In the older eggs no fat was found. It is, however, questionable whether this is not to be attributed to fat loss by solution. Regularly the inner shell of the eggs was entirely blackened by osmic acid. The reaction is, however, too nonspecific for definite proof of its lipoid nature.

SUMMARY

1. Quantitative data are presented concerning the occurrence of dry substance, inorganic substances, nitrogen, glycogen and ether extract in the females of *Macracanthorhynchus hirudinaceus*.

2. Quantitative determinations of dry substance, glycogen and ether extract in different parts of the body are given.

3. A qualitative analysis of the inorganic substances and the polysaccharides found is presented.

4. The results of a quantitative analysis of the ether extract are given.

5. A detailed study is presented of the glycogen and fat deposition, with help of differential staining methods.

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A COMPARATIVE STUDY ON THE BEHAVIOR OF FOUR SPECIES OF PLEUROLOPHOCERCOUS CERCARIAE

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INTRODUCTION

The term *Cercaria* is frequently used in a generic sense, in combination with specific names, to designate caudate larvae of the digenetic trematodes. Although the study of life cycles has received much attention in recent years, the adults and larval stages of relatively few species have been experimentally correlated. Hence it is convenient to describe larval trematodes as species of *Cercaria* although this term is obviously supra-generic in rank.

In studying cercariae, the differentiation of species has been based almost entirely on morphological characters. It is frequently difficult to detect morphological differences in closely related species. However, marked differences in the behavior of such larvae may be noted in certain groups and a study of their swimming habits and reactions to various stimuli may be the deciding factor in differentiating species.

The present study deals with the behavior of four species of cercariae belonging to a restricted group, the Pleurolophocerca Group (Sewell, 1922), and emphasizes the value of careful physiological observations in differentiating members of this group.

Many investigators have noted phototactic responses and swimming habits of cercariae but, until recently, have recorded only brief notes and references to their behavior. Cort and Talbot (1936) utilized swimming habits and resting positions in studying four species of schistosome cercariae, *Cercaria elvae* Miller, 1923; *C. physellae* Talbot, 1936; *C. stagnicolae* Talbot, 1936; and *C. douthitti* Cort, 1914. Although the first three of these are very similar morphologically, Cort and Talbot observed that careful study of their swimming habits made it possible to identify them with a low-power lens. They also concluded that *C. elvae* and *C. ocellata* La Val., 1855, are not synonymous as suggested by several authors, since differences in the position of body and tail while resting could be correlated with differences in body size.

Miller (1926) studied the swimming behavior of five species of larval trematodes infecting the marine snail, *Cerithium litteratum* Born. He found their behaviors so characteristic that each could be identified by inspection with the unaided eye. Of this group, the pleurolophocercous form, *Cercaria P* Miller, 1926 (=*C. floridensis* McCoy, 1929), is very similar in behavior to two of the species included in the present study.

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A consideration of the varied behaviors of larval trematodes suggests the question as to whether these behaviors are adaptations which aid in the completion of life cycles. Miller and McCoy (1930) noted that *C. floridensis* swam in response to shadowing and concluded that this behavior was effective in disseminating the larvae over a greater area, but not necessarily bringing them in contact with the object casting the shadow. Under strong illumination, fish confined near the surface of the water became more heavily infected than those confined near the bottom of the aquarium, but the opposite was true when the experiments were conducted in the dark room.

Miller and Mahaffy (1930) studied the reactions of *C. hamata* Miller, 1923, to photic and mechanical stimuli and found that this species would respond to more stimulations per minute, and swim for a longer period after mechanical than after shadow stimulation. Spontaneous swimming movements were of still less frequency and duration.

Beverlander (1933) studied the behavior of cercariae of *Bucephalus elegans*, noting the effects of varying light intensities and temperatures on their response to shadowing. His results indicated that the reaction time of the cercariae varied inversely with both temperature and luminous intensity, the relationship being hyperbolic rather than linear. Using the same species, Folger and Alexander (1938) studied the effects of mechanical shock and incomplete recovery on the reaction time. Folger and Alexander used the term "reaction time" to designate the interval of time from the application of the stimulus until the end of the response. Since the term is generally employed in a different sense, the use of another term, *e.g.*, reaction period, would have been less confusing.

MATERIALS AND METHODS

All four of the species of pleurolophocercous cercariae studied were obtained from *Goniobasis semicarinata* (Say), collected in Madison County, Kentucky. This mollusk is very hardy and can be kept alive in the laboratory for several months with little difficulty. The snails were placed in finger bowls and observed frequently under a binocular microscope until cercariae emerged. Infected snails were then isolated and the cercariae removed at regular intervals. The age of the larvae was calculated from the time of emergence.

General observations on the swimming movements were made with a number of cercariae isolated in a finger bowl or Syracuse dish. The comparative observations were made on individual cercariae placed in observation cells made of pieces of glass mounted on a slide with xylodamar to form a chamber $7 \times 10 \times 1$ mm.

All observations were made in a dark room using a binocular microscope with the stage mounted in a vertical position. A Spencer microscope lamp with a 100-watt bulb and a diaphragm opening of 4 cm was

used as a source of light. The light was placed 70 cm from the observation cell, giving a maximum luminous intensity of 18–20 foot candles which could be reduced as desired by means of a rheostat. Shadow stimuli of the desired frequencies were made by interrupting the beam of light with a piece of cardboard. Observations were made at a room temperature of approximately 25° C.

To test the effect of galvanic current on behavior, copper electrodes were placed in a finger bowl of tap water containing the cercariae and the snail host. The mollusks were very sensitive to the electrical stimulation and served as a test of the circuit.

OBSERVATIONS

I. *General Behavior.* During the first several hours after emergence, the cercariae decrease in activity as indicated by the rate of swimming movements. This decrease is most rapid in *C. semicarinatae.* All species may live for a considerable time after spontaneous swimming movements cease. *C. vogeli* loses its tail at about 36 hours of age but may remain irritable for an additional 24 hours. After decaudation the body contracts and extends more or less vigorously in response to mechanical stimulation. This response diminishes gradually with increase in age until it becomes only a slight movement of internal structures without noticeable changes in body shape. In species which swim in response to shadowing, it was found that a sudden change of illumination from full to half the intensity would effect a response, but a sudden increase of illumination caused no visible response in any of the species observed.

Each of the species studied is quite sensitive to mechanical stimulation. In a group of cercariae in the same container, concerted responses may be caused by the swimming of one of the group. A jet of air directed against the surface of the water also causes concerted swimming movements and *C. constrictovesica,* at a depth of one to two centimeters, will respond to breaking the surface film of the water.

Before giving the results of comparative observations, the behavior of each species may be described.

Cercaria vogeli Cable, 1935b

Cercaria vogeli, the largest of the species observed, is a relatively powerful swimmer. Each swim is very short and, although erratic, is generally upward and toward the source of light. When the cercariae collect at the side nearest the light and the dish is rotated 180 degrees, they immediately swim across the container in an intermittent, jerky manner quite characteristic of the species. Under ordinary laboratory conditions, *C. vogeli* lives approximately 60 hours. In most cases, however, the tail is lost at about 36 hours of age. No significant change in the

length of the swimming movement was observed during the first ten to twelve hours after emergence.

The response of *C. vogeli* to a decrease in the intensity of illumination (*i.e.*, shadows) is very positive and follows the application of the stimulus without an observed reaction time. A cercaria shadowed at the rate of 12 to 15 times per minute will respond to practically every stimulation for two minutes or longer. The swim begins and ends abruptly. During the rest period, the larva sinks slowly, body downward, through the water. The position of the tail is such that a line drawn from its tip to base forms approximately a right angle with the long axis of the body, while the angle between the proximal part of the tail and the body is considerably greater.

Cercaria opacocorpa Cable and Wheeler, 1939

The resting position and the swimming movements of *C. opacocorpa* resemble very closely those of *C. vogeli*. The swim begins and ends abruptly. The cercariae are photopositive and the rate of progression toward a light source is comparatively rapid. Six individuals were observed to travel an average of 3.8 cm per minute, only five seconds of this time being spent in actual swimming. The rate of progression can be accelerated by repeated shadow stimulation. The cercariae lose their irritability to shadow stimuli at approximately 18 hours of age, but will respond to touch six to eight hours longer.

Cercaria constrictovesica Cable and Wheeler, 1939

Cercaria constrictovesica is an intermittent swimmer but each swimming movement is considerably longer than that of either *C. opacocorpa* or *C. vogeli*. From a resting position on the bottom of the container, the cercariae swim almost vertically at first and then toward the source of light. If the larvae are suspended in the water, the path of movement cannot be predicted; but the general direction is toward the region of strongest illumination. Due to the longer swimming time, the path is more easily observed in *C. constrictovesica* than in *C. vogeli* or *C. opacocorpa* although all three species swim in a somewhat similar manner. *C. constrictovesica* differs from other species studied in that it frequently halts near the end of a swimming movement, and then gives another lash or two of the tail. Newly emerged cercariae swim spontaneously often enough to remain suspended in the water, but as they become older settle to the bottom of the dish. The length of life is approximately 40 hours.

Cercaria constrictovesica is photopositive, the larvae collecting in the part of the container that is most strongly illuminated. The response to a sudden decrease in illumination is not definite in the younger cercariae. There is sometimes a response to the first shadow stimulus, but unless

very long intervals of time are allowed for recovery, this species does not respond to succeeding stimuli.

Cercaria semicarinatae Cable and Wheeler, 1939

As far as swimming habits are concerned, *C. semicarinatae* might be mistaken for one of the many xiphidiocercariae, since it swims almost constantly during the first few hours after emergence. The rest periods of young cercariae are very brief but become progressively longer as the age increases.

There were no observed phototactic reactions in this species. While the cercariae rested, neither the shadow stimulus nor an increase in intensity caused resumption of the swimming. Miller (1926) reported that *Cercaria D*, a small gymnocephalous form, responded to a shadow stimulus by immediate cessation of swimming. After 5–15 seconds the larvae again swam spontaneously, or, after an interval, swimming could be induced by a sudden increase in the intensity of light. No results of this type were obtained with *C. semicarinatae*.

The swimming of this species is very erratic, the direction being changed frequently. The path of swimming changes without any observable external stimulus. *C. semicarinatae* is smaller, more active, and lives for a shorter time than any of the species studied. The swimming movements were greatly reduced in 12 hours and nearly all cercariae died in 15 to 18 hours.

TABLE 1.—*Relation of rate of spontaneous swimming movements to age of cercariae, as determined by average number of swims per minute (50 observations on each species at each age)*

	C. vogeli			*C. constricto-vesica*			*C. opacocorpa*		
Age	Mean	P.E.	V	Mean	P.E.	V	Mean	P.E.	V
Less than 2 hrs.	15.2	± 0.45	31	6.1	± 0.20	34	7.5	± 0.28	38
2–4 hrs.	8.5	± 0.45	55	5.0	± 0.16	34	6.2	± 0.21	36
4–6 hrs.	13.9	± 0.77	58	4.5	± 0.11	26	7.3	± 0.21	30
6–8 hrs.	8.2	± 0.48	63	3.1	± 0.21	72	6.2	± 0.29	49

P.E. = probable error. V = coefficient of variation.

II. *Relation of rate of spontaneous swimming movements to age of cercariae.* Each mean, probable error, and coefficient of variation, as entered in Table 1, was derived from fifty observations (five individuals used, 10 observations on each). The activity of all cercariae decreased with an increase in age. The spontaneous swimming of *C. vogeli* was considerably more irregular than that of the other species. In certain individuals, periods of frequent swimming alternated with intervals during which there were relatively few swims. The time between successive active periods became greater as the cercariae aged. The spontaneous activity of *C. constrictovesica* decreased consistently as the age increased.

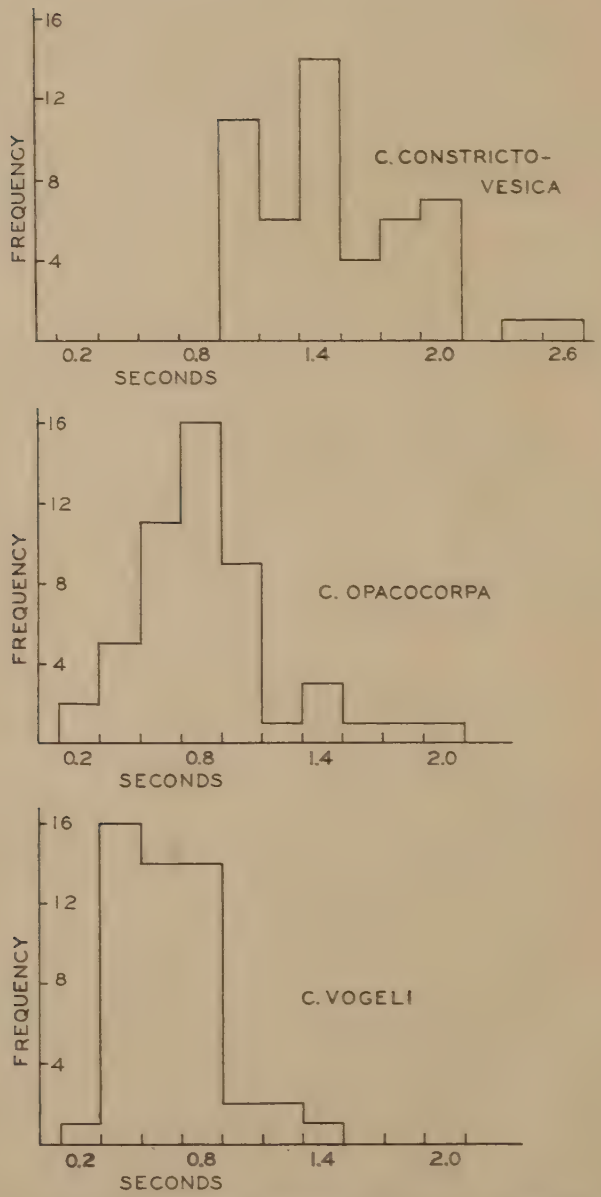

FIG. 1. Duration of swimming movements. Each curve represents 50 observations, 10 on each of 5 cercariae. All larvae were less than 1 hour of age.

With the exception of the 2–4 hour age group, the frequency of swimming movements was less than half that of *C. vogeli* at corresponding ages. The rate of spontaneous swimming movements of *C. opacocorpa* did not differ greatly from that of *C. constrictovesica*, but these species could be differentiated readily by observing the length of swimming movements (Fig. 1).

III. *Duration of swimming movements.* Fig. 1 represents the duration of swimming movements of *C. vogeli*, *C. constrictovesica*, and *C. opacocorpa*. It was observed that *C. constrictovesica* differed significantly from the other species in this respect. In fact, the difference was sufficiently great to distinguish *C. constrictovesica* without resorting to quantitative determinations. *C. vogeli* and *C. opacocorpa* which are very different in respect to morphology and rate of spontaneous swimming, did not differ significantly in the duration of their swimming movements. In Fig. 1 it was necessary to omit observations on *C. semicarinatae* because of the extremely long duration of its swimming movements. Individuals of this species have been observed to swim continuously for as long as 29 minutes. The rest periods were very short in younger specimens. Larvae 4–6 hours of age swam very irregularly, the duration of the swimming movements varying from 15 seconds to 15 minutes with rest periods of 5 to 10 seconds. There seemed to be no correlation between the duration of a swim and the duration of the succeeding rest period.

IV. *Effect of shadow stimulation on rate of swimming movements.* Shadow stimulation was effective in increasing the rate of swimming in all species (Table 2) when compared to the spontaneous rate of swim-

TABLE 2.—*Effect of shadow stimulation (30 stimuli per minute) on rate of swimming movements. Each entry represents the average swims per minute obtained by five observations on different individuals.*

Age	*C. vogeli*	*C. constricto-vesica*	*C. opacocorpa*
Less than 2 hrs.	23.2 ± 0.50	8.2 ± 0.52	8.6 ± 0.45
4–6 hrs.	18.6 ± 1.65	6.4 ± 0.48	13.4 ± 0.67
6–8 hrs.	16.2 ± 1.35	9.8 ± 0.61	16.8 ± 2.47

ming (Table 1). *C. constrictovesica* seemed to be least affected, while *C. vogeli* and *C. opacocorpa* were more sensitive to shadow stimulation. With an increase in age, however, the response of *C. vogeli* decreased while that of *C. opacocorpa* increased.

Fig. 2 includes the results of continuous observations on *C. vogeli*, *C. constrictovesica*, and *C. opacocorpa* at an age of one hour or less, five individuals of each species being used. The numbers of spontaneous swimming movements of the cercariae were observed for a period of one minute. During the second minute (A_2), the cercariae were subjected to shadow stimuli at a rate of 30 per minute and the number of swims ob-

served. During the following three minutes, the number of spontaneous swims per minute was recorded, the larvae then being subjected to shadow stimuli for one minute at a rate of 60 per minute (B) and the number of swims recorded. Again spontaneous swims per minute were observed for three consecutive minutes following which the cercariae were shadowed for one minute at a rate of 30 per minute (A_2). Finally, two minutes after the last period of stimulation, another determination of the number of spontaneous swims was recorded for comparison with the initial readings.

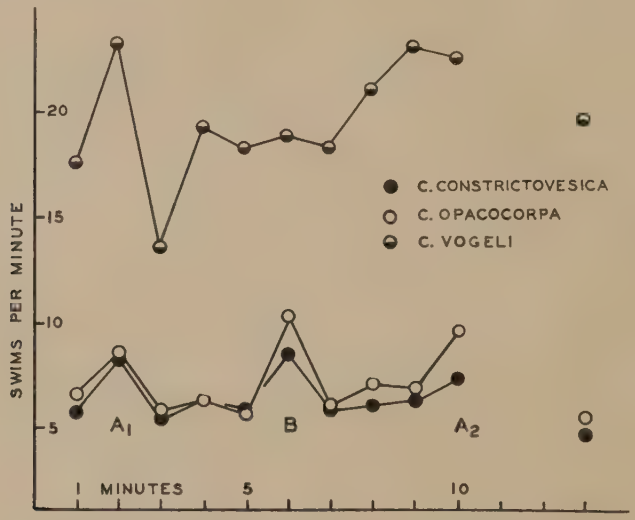

FIG. 2. Effect of shadow stimulation on the rate of swimming movements during consecutive minutes. Each point on the curves represents the average of observations on five individuals. Shadow stimuli at 30 per minute were applied at A_1 and A_2, stimuli at 60 per minute at B. No readings were made for the 11th or 12th minute. Cercariae one hour or less of age.

Shadowing at a rate of 30 per minute was more effective in increasing the number of swims of *C. vogeli* than shadowing 60 times per minute. Since the numbers of spontaneous swims following the period of stimulation were normal within three minutes, it was concluded that the period of shadowing at two-second intervals did not affect the response to stimulation at the one-second interval. The results of the second period of stimulation at 30 per minute (A_2) were comparable to those of the first period of shadowing at the same rate except it will be noted that the average rate of spontaneous swimming of *C. vogeli* was slightly higher during the minute preceding the third period of shadowing than during that period of stimulation. This behavior may be due to a delayed reaction to

the second period of stimulation (one-second intervals) during which the larvae swam at approximately the normal rate.

As indicated in Table 1 and in Fig. 2 the rates of spontaneous swimming of *C. opacocorpa* and *C. constrictovesica* were not significantly different. These species also responded to shadow stimulation in a similar manner, and, unlike *C. vogeli,* responded as much to shadowing at one-second intervals as at intervals of two seconds.

V. *Response to galvanic current.* Galvanic currents of 1.5 and 6 volts were passed through water containing *C. vogeli* and *C. opacocorpa* along with the snails from which they had emerged. The mollusks were very responsive even to the weaker current and rapidly withdrew into their shells while the cercariae were apparently unaffected. The possibility of injury was precluded by the fact that spontaneous swimming movements continued in a normal manner.

VI. *Time of emergence.* In a preliminary experiment, snails known to be infected with *C. vogeli* and *C. opacocorpa* were isolated and placed under constant observation for a 24-hour period. The snails used had been kept in the laboratory for three weeks. The cercariae were counted at the end of each two-hour period.

On April 23, the larvae emerged in the late afternoon and evening (3:00–11:00 PM) with a maximum rate at 3:00–4:00 o'clock. On May 4, further observations were made on three snails infected with *C. vogeli,* three with *C. constrictovesica,* and one with *C. opacocorpa.* All of these snails had been in the laboratory for five weeks. In this case, nearly all the cercariae emerged between 10:30 PM and 3:00 AM with a maximum rate for all species at 1:00–2:00 AM. The same snails were observed on succeeding nights and approximately the same emergence time was noted for all species.

In the course of the above observations it was noted that the snails infected with *C. vogeli* harbored also *C. constrictovesica* and *C. kentuckiensis* Cable, 1935a, indicating that triple infections may be fairly common.

DISCUSSION

Distinguishing the species of pleurolophocercous cercariae studied is relatively simple when based upon comparative data of (1) the number of spontaneous swims per minute, (2) the nature and duration of each spontaneous swim, and (3) the relative effect of shadow stimulation.

Cercaria opacocorpa and *C. semicarinatae,* the most similar morphologically, represent the extremes in behavior. *C. opacocorpa* swims intermittently and for an average time of less than one second while *C. semicarinatae* swims nearly constantly for the first few hours after emergence. Of the four species studied, *C. vogeli* and *C. opacocorpa* differ most in size and structure, but have the most similar types of behavior.

Considerable individual variation was observed which may have been

due to one or a combination of the following conditions. Since the cercariae were isolated hourly and the observations begun immediately thereafter, certain individuals of the youngest age group may have been only a few minutes old when the experiment began, and others almost two hours old by the time the observations were completed. It is possible that the change from the body fluids of the host to the water medium may have had an effect upon the behavior of the cercaria, an effect which disappears after the animal becomes adapted to the water. The results indicate that individuals of each species possess inherent physiological differences which cannot be explained on the basis of variation in time between emergence of the larvae and the time of observation. Such differences do not lend themselves readily to experimental analysis. However, a possible explanation of the observed variations lies in the fact that pleurolophocercous cercariae escape from the rediae in an immature condition and complete their development in the tissues of the host: some of the larvae may be more mature than others when they emerge from the snail. A few cercariae upon emergence did not behave in a normal manner, sometimes appearing very weak and dying in a short time.

According to Cable (1938), *C. vogeli* emerges from the snail almost exclusively in the late afternoon and early evening. His observations, which do not agree entirely with those of the writer, were made in the late summer on recently collected material, while the snails used in the present study were collected in March and kept in the laboratory for three to five weeks. During this time, cercariae emerged more or less regularly. When a specific study of emergence was made, results agreeing with those of Cable were obtained on one occasion. In subsequent experiments, however, a relatively small number of cercariae escaped during the late afternoon, the majority of them emerging during the early morning, sometimes starting about midnight. The rate of emergence was not uniform; large numbers of larvae of all species observed usually escaped in a comparatively short time, a single snail frequently shedding 200–300 cercariae in an hour. Emergence was not correlated with the activity of the snail since the cercariae often escaped while the snail was relatively inactive, and none emerged during periods of great activity of the host. It was noted, however, that the activity of the snail frequently decreased after the escape of large numbers of cercariae in a short interval. Intermittent emergence may be associated with a mechanical release of the cercariae from the tissues of the snail. It is conceivable that when one cercaria breaks through the retaining tissue it might provide a means of egress from an interlobular space containing several cercariae, which may vary in the degree of maturity. These cercariae may escape from the space thus opened in a comparatively short time.

SUMMARY

Four species of pleurolophocercous cercariae from *Goniobasis semicarinata* (Say) were observed and comparative studies on their behavior at various ages were made. Specific differences in the frequency and duration of spontaneous swimming movements and responses to shadow stimulation are demonstrable. The desirability of comparative observations of behavior as a supplement to morphological studies is stressed.

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THE INTESTINAL PROTOZOA OF MAN IN MIDWESTERN CANADA

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Fantham and Porter (1930) demonstrated that the population of Montreal, Canada, harbored numerous species of intestinal protozoa and helminths, and that many of them showed high infection incidences. This study, along with an earlier survey carried out by Porter (1934), constitutes the only inquiry into the problem of human intestinal parasites in Canada. The present paper presents the results of a survey for intestinal parasites of man carried out in Saskatoon, Saskatchewan, in the summer of 1938.

Three different population groups were examined: (1) a clinical group comprising hospitalised and non-hospitalised patients of both sexes, with ages ranging from 5 to 70 years; (2) a healthy group including male college students and student nurses; (3) a group of children from an orphan asylum, representative of an institutionalised group in the lower age bracket, with ages ranging from 2.5 to 18 years, but with the greatest number between 7 and 12 years of age.

Stools were obtained not later than 24 hours after passage and were examined by two technics: (1) the direct fecal smear in physiological salt solution, unstained and iodine-stained; (2) the zinc sulphate centrifugal-flotation technic (Faust, *et al.*, 1938). Where identification was doubtful these technics were supplemented by the haematoxylin-stained film. Only one stool was examined from each individual.

RESULTS

Stools of 254 persons were examined: 97 or 38.2% were found positive for parasites. Only protozoa were found, although *Ascaris*, *Enterobius*, whipworms, and several species of tapeworms are known to occur in the area. The species diagnosed included *Endamoeba histolytica*, *Endamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Dientamoeba fragilis*, *Giardia lamblia*, and *Chilomastix mesnili*. In the institutionalised group 28.2% of the infected persons showed triple infections, and 32.6%, double infections. In the two groups of the general population only 8.3% of the infected persons showed double infections. Table 1 presents the incidence of each species of protozoa in the three groups according to sex. Group IV comprises the combined results of groups I and II, and is taken as more representative for the general population

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TABLE 1.—*Distribution of the different species of intestinal protozoa in the Saskatchewan population*

Group		Number examined	Infection rate		*E. coli*		*E. histolytica*		*E. nana*		*I. bütschlii*		*D. fragilis*		*G. lamblia*		*C. mesnili*	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I. Clinic patients	males ..	104	30	29	23	22	1	1	7	7					1	1	4	4
	females .	45	10	22	10	22	0	0	1	2					0	0	1	2
	total ...	149	40	27	33	22	1	1	8	5					1	1	5	3
II. Healthy persons	males ..	33	11	33	5	15	1	3	4	12			1	3	1	3	1	3
	females .	25	12	48	8	32	0	0	1	4			0	0	3	12	0	0
	total ...	58	23	40	13	22	1	2	5	9			1	2	4	7	1	2
III. Orphan asylum cases	males ..	29	21	72	15	52	8	28	10	35	3	10			3	10		
	females .	18	13	72	6	33	3	17	6	33	0	0			6	33		
	total ...	47	34	72	21	45	11	23	16	34	3	6			9	19		
IV. General population (gr. I & II)	males ..	137	41	30	28	20	2	2	11	8			1	1	2	2	5	4
	females .	70	22	31	18	26	0	0	2	3			0	0	3	4	1	1
	total ...	207	63	30	46	22	2	1	13	6			1	0.5	5	2	6	3

because of the greater number of persons it contains. The inclusion of the clinical group I is considered justifiable because only a small percentage of the patients were suffering from intestinal ailments. A study of the table draws attention to the considerably higher infection rate for all species of protozoa in individuals of the institutionalized group than in those of the general population. This is particularly noticeable for *E. histolytica*. The general infection rate was approximately the same in males and females, although certain species occurred more frequently in one sex than in the other.

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MYXOSPORIDIA FROM TIDE POOL FISHES OF CALIFORNIA

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In tide pools at Santa Barbara, California, eight distinct species of fish and several hybrids have been examined for myxosporidia, and seven different species of these protozoan parasites have been found. Two of the latter were described by the writer in 1938. A study of the parasites and their hosts might be used to throw light upon the problem of host-parasite specificity.

All of the following descriptions and measurements were made upon living material. Kudo's (1930) method of using two coverslips and a depression slide was employed. Aid was gratefully received from Professor Percy Spencer Barnhart in the identification of two of the fish.

Leptotheca compressa n. sp.

(Figs. 1–2)

Specific diagnosis: Genus *Leptotheca.*

Trophozoite oval and flattened, 17 by 22.5 microns (Fig. 1). Ectoplasm narrow and clear; endoplasm finely granular. No movement or pesudopodia observed. Disporous.

Spore roughly pyramidal, flattened, breadth 12.2 microns, sutural diameter 10.5 microns. Two polar capsules both at top of pyramid, 3.3 by 4 microns. Coiled polar filaments and capsulagenous cells easily visible in living spore. Sporoplasm very lightly granular, contains two small nuclei and is withdrawn from area about polar capsules (Fig. 2).

Host: Rimicola eigenmanni (Gilbert).

Location: Urinary bladder.

Locality: Tide pools of Santa Barbara, California.

The most closely related species to the above is *L. sphaerula* described in this paper.

Leptotheca sphaerula n. sp.

(Figs. 3–4)

Specific diagnosis: Genus *Leptotheca.*

Trophozoite roughly egg-shaped, 20 by 28 microns. Endoplasm filled with masses of irregular yellowish-brown bodies of various sizes (Figs. 3–4); ectoplasm not sharply differentiated from endoplasm. Usually only one or two broad, transparent pseudopodia which move very slowly. Usually disporous, seldom monosporous.

Spore spherical, 13 microns in diameter. Two rounded polar capsules at one end, 4.6 by 5 microns (Fig. 4). Polar filaments narrow and tightly coiled. Sporoplasm very lightly granular, fills extra capsular space and contains two nuclei. Suture line indistinct.

Host: Gibbonsia metzi Hubbs.

Location: Urinary bladder.

Locality: Tide pools of Santa Barbara, California.

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Of the described species of *Leptotheca* the most similar to *L. sphaerula* is *L. chagasi* Nemeczek from the kidney tubules of *Leptodactylus* (*Cystognathus*) *ocellatus*. The trophozoite of the latter is larger and possesses long pointed pseudopodia; and the spore is elliptical with one to three polar capsules which measure up to 8.5 microns in diameter. The yellow bodies of *L. sphaerula* suggest the large yellow fat globules of *L. lobosa* Davis.

Sphaeromyxa gibbonsia n. sp.

(Figs. 5–6)

Specific diagnosis: Genus *Sphaeromyxa.*

Trophozoite forms a flat folded plasmodium up to 2 mm. in diameter, white in color. Endoplasm alveolar; ectoplasm not distinct. No pseudopodia or movement observed in mature stages. Each pansporoblast develops two spores all at about the same stage of development at any one time. Spores at periphery are parallel to each other and lie at right angles to plane of surface of plasmodium. Polysporous. Occasionally with the mature plasmodium are a few ameboid forms 25 microns in diameter (Fig. 5). Whether or not the latter are young trophozoites has not been determined.

Spore elongated, curved with rounded ends. Degree of curvature varies, usually slight (Fig. 6). Length (sutural diameter) 27 microns; thickness 5.2 microns. A polar capsule, 10 by 4 microns, at each end. Polar filaments easily observed in living specimens are short, thick, loosely coiled and measure 20 microns in length. Suture line usually at a slight angle to main axis of spore. Sporoplasm clear, fills extracapsular space, with one or two nuclei. Capsulogenous cells distinct.

Host: Gibbonsia elegans elegans (Cooper) and *G. metzi* Hubbs.

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

The most closely related species to the above are *S. hellandi* Auerbach from the gall bladders of *Molva vulgaris, Centronotus gunellus,* etc., and *S. sabrazesi* Laveran et Mesnil from the gall bladders of *Hippocampus brevirostris, Syngnathus acus, Motella tricirratam,* etc. *S. gibbonsia* differs from these species principally in the proportions of the spore and thickness of the polar filament, the latter being much narrower in the European species.

Sphaeromyxa ovula n. sp.

(Figs. 7–8)

Specific diagnosis: Genus *Sphaeromyxa.*

Trophozoite a large, flat, leaf-like plasmodium when mature; diameter up to 800 microns (Fig. 7). No distinction between ectoplasm and reticulated endoplasm. Each pansporoblast develops two spores. Spores in all stages of development. No pseudopodia or movement observed. Polysporous.

Spore oval with rounded ends; length 14 microns, breadth 4.3 microns. Two polar capsules, terminal, oval, 3 by 5 microns. Sporoplasm very finely granular, fills extracapsular space. Polar filaments short, indistinct. Suture line runs in straight line parallel to long axis of spore, indistinct (Fig. 8).

Host: Arbaciosa rhessodon (Rosa Smith).

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

Of the species of *Sphaeromyxa* already described, this parasite is most similar to the type species, *S. balbianii* Thélohan. The trophozoite of the latter differs from that of *S. ovula* in being much larger (up to 4 mm. in diameter) and in possessing clear ameboid ectoplasm. The spore of *S. balbianii* is truncated, striated and possesses very thick filaments.

Trilospora californica n. g., n. sp.

(Figs. 9–13)

Specific diagnosis: Genus *Trilospora.*

Trophozoite (Figs. 9, 10) polymorphic, greatest length 100 microns. Ectoplasm clear and distinct in long pointed pseudopodia; endoplasm very granular, usually with large refractive globules. Slow ameboid movement; pseudopodia numerous. Often a slow change of shape without formation of distinct pseudopodia. Mono- and disporous.

Spore triangular in polar view (Fig. 11); oval in lateral view (Fig. 12). Each side of the triangle is concave. Breadth 16 microns, height 7.2 microns. Polar capsules pyriform (lateral view) 1.5 by 3 microns. Frequently four polar capsules present (Fig. 13). Polar filaments not observed. No distinct sutural ridges.

Host: Gibbonsia elegans elegans (Cooper) and *Typhlogobius californiensis* Steindachner.

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

This parasite was first believed to be an aberrant form of *Leptotheca elegans* (Noble 1938, Fig. 6). Since that time it has been found in only one specimen of *Gibbonsia e. elegans,* but is abundant in *Typhlogobius californiensis.*

This is the only known myxosporidian with three polar capsules, which justifies the creation of a new genus. Its relation to other members of the order Myxosporidia has not been determined since there are no existing families in which it can readily be placed. The type, and only, species is *T. californica.*

Two new hosts for *Leptotheca elegans* have been found. One is *Auchenopterus integripinnis* (Rosa Smith) and the other is *Gibbonsia metzi* Hubbs. The gall bladders of both are infected.

Jameson (1929) described a myxosporidian parasite, *Ceratomyxa gracilis,* from the gall bladder of *Gibbonsia evides* collected at Monterey, California. This parasite has not been found in the species of *Gibbonsia* in southern waters. Jameson, in the same paper, also found *Clinocottus analis* to be parasitized by *Ceratomyxa obesa.* Although *Clinocottus analis* is abundant at Santa Barbara no parasites have been found in it by the writer.

Three fish were found which possessed characteristics of both *Gibbonsia e. elegans* and *G. metzi.* These, therefore, were considered as hybrid species and their gall bladders were found to contain a small variety of *Leptotheca elegans* (average length of spore 11.5 microns). Thus, insofar as tide pool fishes of the genus *Gibbonsia* are concerned,

most species of myxosporidia are best adapted to one species of fish in which there are optimum conditions for the growth and development of the parasite. Among the myxosporidia in tide pool fishes in general there is, on the whole, definite host-parasite specificity in spite of the close association and occasional hybridization among the different species of hosts.

A list of tide pool fishes of California from which myxosporidia have been described, together with their parasites, follows:

Fish	Site of infection	Parasite
Arbaciosa rhessodon	gall bladder	*Sphaeromyxa ovula*
Auchenopterus integripinnis	" "	*Leptotheca elegans*
Clinocottus analis	" "	*Ceratomyxa obesa*
Gibbonsia elegans elegans	" "	*Leptotheca elegans*
" " "	" "	*Sphaeromyxa gibbonsia*
" " "	" "	*Trilospora californica*
Gibbonsia evides	" "	*Ceratomyxa gracilis*
Gibbonsia metzi	urinary bladder	*Leptotheca sphaerula*
" "	gall bladder	*Leptotheca elegans*
" "	" "	*Sphaeromyxa gibbonsia*
Hypsoblennius gilberti	" "	*Ceratomyxa blennius*
Rimicola eigenmanni	urinary bladder	*Leptotheca compressa*
Typhlogobius californiensis	gall bladder	*Trilospora californica*

In a previous paper (Noble 1938) the writer stated that the only myxosporidia reported from the California coast were those described by Jameson and by himself. This is erroneus. Thompson (1916) and Davis (1924) both recorded a myxosporidian parasite in relation to "wormy" halibut of the Pacific coast.

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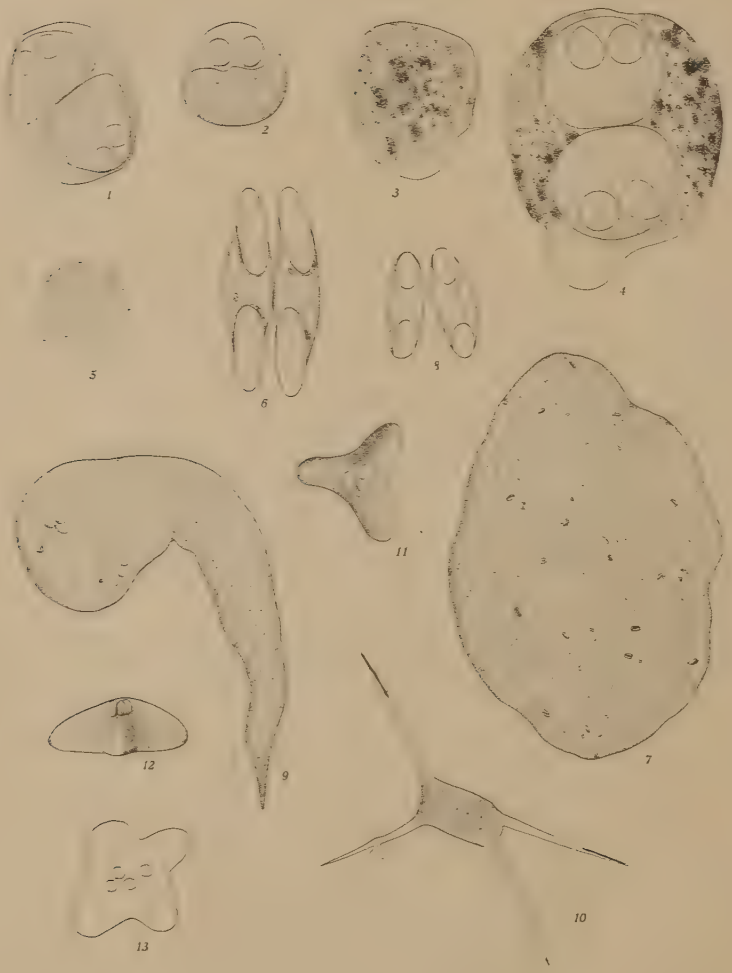
All figures drawn from living material with the aid of a camera lucida. Magnification ×1150 (except Fig. 7 which is ×100).

FIG. 1. Trophozoite of *Leptotheca compressa.*
FIG. 2. Mature spore of *Leptotheca compressa.*
FIG. 3. Young trophozoite of *Leptotheca sphaerula.*
FIG. 4. Mature trophozoite of *Leptotheca sphaerula* with two spores.
FIG. 5. Ameboid forms found with *Sphaeromyxa gibbonsia.*
FIG. 6. Two spores of *Sphaeromyxa gibbonsia.*
FIG. 7. Plasmodium of *Sphaeromyxa ovula.*
FIG. 8. Two spores of *Sphaeromyxa ovula.*
FIG. 9. Large trophozoite of *Trilospora californica* without pseudopodia.
FIG. 10. Small trophozoite of *Trilospora californica* with pseudopodia.
FIG. 11. Polar view of spore of *Trilospora californica.*
FIG. 12. Lateral view of spore of *Trilospora californica.*
FIG. 13. Outline of spore with four polar capsules which is frequently observed with normal spores of *Trilospora californica.*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STUDIES ON STRONGYLOIDES. V. CONSTITUTIONAL DIFFERENCES BETWEEN A HOMOGONIC AND A HETEROGONIC LINE OF *S. RATTI*

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Strongyloides ratti exhibits, as do most if not all other species of *Strongyloides,* alternative modes of larval development. On the one hand, the rhabditiform larval progeny of the parasite may metamorphose outside the body of the host directly into infective filariform larvae or, on the other, into sexually differentiated free-living adults. These in turn produce offspring metamorphosing into infective filariform larvae morphologically indistinguishable from those produced directly. These two modes of larval development are known as homogonic or direct, and heterogonic or indirect, respectively.

There is essential agreement among many investigators who have studied various species of *Strongyloides* that the direction of larval development is predetermined, i.e., it is an inherent or constitutional function of the egg from which the larva originates. Nevertheless another point of view still prevails regarding the fundamental factors involved in determining the direction of larval development. Periodically, as well as recently (Beach, 1936), it has been contended that the mode of larval development is not determined by the inherent constitution of the egg but by environmental influences which the young larvae encounter during their period of free-living development.

The implications of this latter hypothesis are clear, and insofar as they may apply to *Strongyloides ratti,* are subject to test. If it is true that elements in the fecal environment influence and thus determine the mode of larval development followed by the offspring of *S. ratti,* then it necessarily follows that all *S. ratti* are constitutionally equivalent insofar as this specific point is concerned. Thus any newly hatched larva would potentially be able to develop in one direction or the other depending on the environmental influences to which it is exposed. Consistently demonstrable differences, referable to constitution, should not, theoretically, appear.

The present study is a comparison of a line of singly established, *homogonically* derived *S. ratti* maintained serially in the laboratory rat (Graham, 1935a, 1936) and a line of singly established, *heterogonically* derived *S. ratti,* maintained in the same way. Certain characteristics of the latter line have been briefly mentioned in previous reports (Graham, 1938a and c, 1939).

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MATERIALS AND METHODS

The technical procedures with the *homogonic* line have been described previously (Graham, 1936, 1938a and b, 1939). Those employed with the *heterogonic* line have been essentially the same, differing principally in that all fecal cultures (i.e., the 24-hour fecal output from each rat harboring a single, heterogonically derived *S. ratti*) were isolated in the Baermann apparatus after an incubation period of only 24 hours, instead of the 48- or 72-hour period used for fecal cultures from the homogonic line. This shorter incubation period was used to ensure perfect differentiation between the larvae of direct development and the larval progeny of the bisexual generation, which are frequently well developed after 48 hours' incubation. After 24 hours' incubation only small numbers of the females of heterogonic development have started to oviposit while most larvae of direct development have reached the filariform stage, although they frequently have not undergone the last ecdysis in the free state.

To initiate the heterogonic line and to maintain it in series, adult females of the bisexual generation were selected from those secured by Baermann isolation; those containing obviously developing ova being selected preferentially (many of these females have already copulated after an incubation period of 24 hours). These females were then placed singly in feces from a helminth-free rat and incubated for 72 hours before re-isolating. The infective filariform larvae of heterogonic development thus obtained were then employed to infect helminth-free rats with a single larva each. Thus between each parasitic generation in the indirect series there was interpolated a free-living bisexual generation. Obviously such a line is pure, and unity of maternal ancestry is assured in both the parasitic and free-living generations.

The larva used for the establishment of the first parasitic generation in the heterogonic line was obtained from a single female of indirect development selected from a stock culture isolation. This stock culture, predominantly homogonic in type, was the continuation (through a number of generations) of the culture from which filariform larvae of direct development were selected in January, 1935, for the establishment of the first parasitic generation in the homogonic line (Graham, 1936, p. 74).

Attention is directed to the fact that a highly inbred strain of laboratory rats was used. The rats, infected with single parasites from the two lines under comparison, were housed in the same room, fed the same rations, and cared for as uniformly as possible in all respects. The infections, considering a rat and its single *S. ratti* parasite as a unit, differed only in that in one case the parasite was of homogonic origin and derived from a single organism; in the other the parasite, similarly derived from a single worm, was of heterogonic origin.

RESULTS

The comparison of single *S. ratti* from these two lines has shown certain marked differences, some being apparent almost from the first patent day. Four are clear-cut and obvious: a fifth is deserving of mention, although it is not comparable to the other points of difference. These differences are in: (1) the relative yields of direct and indirect progeny, (2) the reproductive rate (fecundity), (3) the length of life (as judged by the cessation of reproduction), (4) the frequency of production of free-living males of the bisexual generation, and (5) the infection rate (i.e., percentage of rats exposed to one larva each which developed fertile infections).

1. Differences in the Relative Yields of Direct and Indirect Progeny

When study of the heterogonic line was initiated, the first distinctive feature setting it apart from the *S. ratti* of the homogonic line was the predominance of adults of indirect development among the progeny obtained. The difference in this respect so greatly exceeded the well-known idiosyncrasies of *S. ratti* from the homogonic line that no doubt existed concerning their essential dissimilarity.

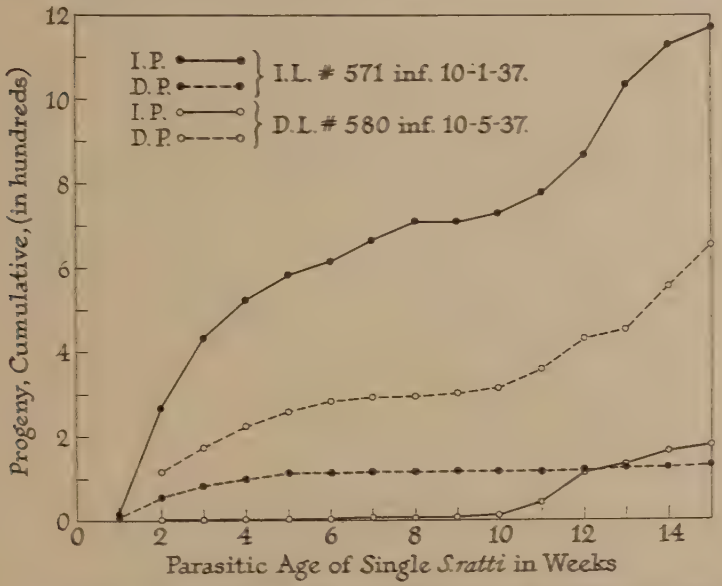

FIG. 1. The cumulative yield of indirect (adult) and direct (larval) progeny (designated respectively by I.P. and D.P.) from a single *S. ratti* of heterogonic origin (No. 571) and a single *S. ratti* of homogonic origin (No. 580). The difference in the relative yields of the two types of progeny under comparable conditions in these two lines of the parasite is clear.

Exemplifying the relative yields of progeny of the two developmental types, the cumulative progeny curves from a single parasite, No. 580, of the homogonic or "direct" line and a single parasite, No. 571, of the heterogonic or "indirect" line are shown in Fig. 1. The predominant production of indirect progeny by the parasite from the heterogonic line is characteristic of all *S. ratti* in this line. On the other hand the parasite from the homogonic line, No. 580, produced predominantly larvae of direct development. This is characteristic of the parasites in this line. It is to be recalled that these *S. ratti* (Nos. 571 and 580) began their parasitic existence only four days apart. They were established in young female rats from a highly inbred line which were fed uniform rations and subjected to as nearly identical technical procedures as was possible. In view of these facts it is virtually impossible to account for the great difference in the rate of production of adult progeny of indirect development on the basis of the environment encountered by the young rhabditiform larvae in the fecal cultures. While it is not possible to conclude that the

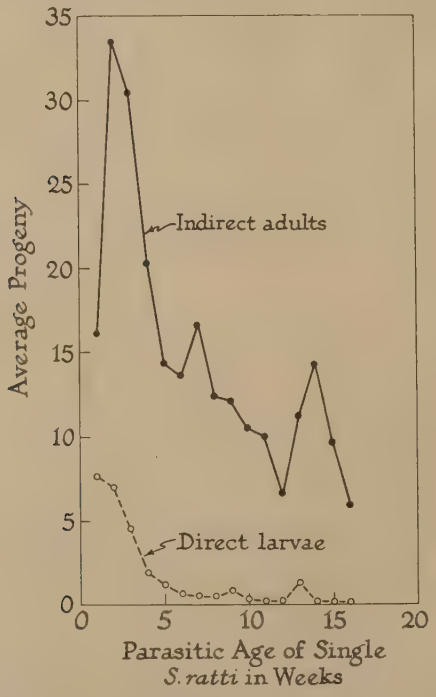

FIG. 2. Average numbers of free-living adults and direct development larvae in the progeny secured from 18 single *S. ratti* parasites of heterogonic origin, as observed in cultures made from 24-hour fecal collections from the rat host.

fecal environment is without influence on the young offspring of *S. ratti,* it is clear that in these two lines the crucial difference is one of inherent constitution.

The disproportion in the relative yields of the two progeny types from the two lines may be shown in another manner. In Fig. 2 the average daily yield of each type of progeny calculated by weeks of parasitic age is shown for 18 parasites of the heterogonic line. A total of 1095 daily cultures is involved from which were obtained 22,279 progeny. The curve of the predominant progeny type, i.e., indirect, is similar to that noted for the predominant progeny type (direct larvae) in the homogonic line (Graham, 1938a, Fig. 2, p. 228). In both of these lines, *S. ratti* produces maximal numbers of progeny during the second week of parasitism. For the next month the decline in progeny numbers is rather abrupt and then becomes more gradual. Thus there is an almost exact reversal in the relationship between progeny types in these two lines. This is solid evidence of their dissimilarity. It may be noted that production of the subordinate progeny type (larvae of direct development) in the heterogonic line is concentrated mainly in the first month of parasitism, whereas the subordinate progeny (free-living adults) in the homogonic line (Graham, 1938a, Fig. 2) were produced at a fairly uniform rate throughout the reproductive life of the parasite.

2. *Differences in the Reproductive Rate (Fecundity)*

From a comparison of the two curves mentioned in the preceding paragraph it is obvious that the reproductive rate in the heterogonic line is approximately 50 per cent higher than in the homogonic line. In order to show this difference with greater accuracy, the data from which the curves of Fig. 2 of this paper and Fig. 2, Graham, 1938a, were prepared have been reorganized and incorporated in Fig. 3. The curves shown are the cumulative progeny yields from the two lines, the weekly increment in each instance being based on the average progeny yield from patent culture days.

The accelerated reproductive rate in the heterogonic line is apparent even in the first week of parasitism, and the distinction between the two lines becomes greater as the parasites increase in age. At the end of 16 weeks, *S. ratti* from the heterogonic line have reached a cumulative level not attained by the *S. ratti* of the homogonic line until 13 weeks later.

It may be objected that the data from the homogonic series antedated those from the heterogonic series and hence do not afford an equitable basis for comparison. On this point it is only necessary to note that over the period where precise calendar comparability between the two lines was obtained, marked differences were obvious. When the data from the comparable homogonic series were analyzed on the same basis as those

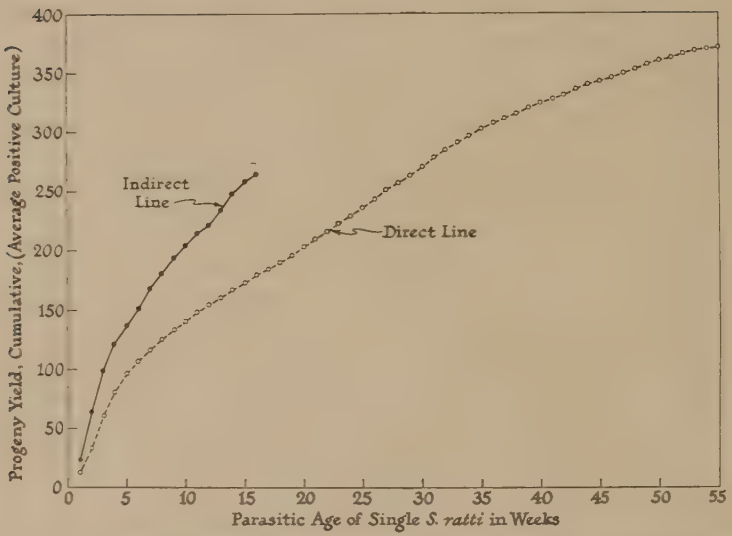

FIG. 3. The cumulative progeny yield from 18 *S. ratti* of heterogonic origin (indirect line) and 116 *S. ratti* of homogonic origin (direct line). Weekly accumulations for each curve are based on the average yields of positive cultures. In the indirect line 1095 culture days yielded 22,279 progeny; in the direct line 5118 culture days yielded 54,867 progeny.

herein employed, the differences in the reproductive rate between the two lines under comparison were somewhat greater than those shown in Fig. 3 in this respect. Evidence of this is shown in Table 1 where 1463 culture days in the homogonic line yielded a total of 9690 progeny, whereas 921 culture days in the heterogonic line yielded 17,680 offspring. Hence it is clear that the higher reproductive rate in *S. ratti* from the heterogonic line has a functional basis of inherent constitutional difference.

3. Differences in the Length of Reproductive Life

Concerning the length of reproductive life in single *S. ratti* of the homogonic line, it was stated (Graham, 1935b) that "the bulk of the parasites reached the negative phase or showed a marked decrease in the number of progeny in culture, in from 2 to 4 months." As the study of this line continued and additional data were obtained, this concept has not been subject to essential modification. To date, approximately 50 per cent of the infections have ceased reproduction within 4 months, and within 6 months 80 per cent were post-patent.

In the heterogonic line a distinctly different condition prevails. Although the number of single *S. ratti* infections in this line is small in comparison with the number in the homogonic line, a much shorter repro-

ductive life was clearly indicated. Most of the *S. ratti* in the heterogonic line reached the post-patent period in about 3 months. Eleven infections for which good end-points, i.e., at least 30 consecutive negative daily cultures, were obtained, produced progeny for from 5 to 15 weeks. The average length of reproductive life was 11 weeks. Only two infections have been observed which persisted for as much as 16 weeks. It seems improbable on the basis of present experience that the average length of reproductive life in this line will deviate greatly from 11 weeks.

Thus the discrepancy in the length of the two curves shown in Fig. 3 is due to a difference in the length of reproductive life of the *S. ratti* in the two lines under comparison. As a matter of fact one might find justification on *a priori* grounds for anticipating that in reproduction an accelerated rate and a shortened life would go hand in hand, as appears to be true in this case.

4. *Differences in the Frequency of Production of Males of the Free-living Bisexual Generation*

Although the difference in the relative production of indirect adults among the progeny from the two lines is great, the difference in the rate of production of free-living males is extreme. This may be seen from the data in Table 1. In the homogonic line, a free-living male occurred

TABLE 1.—*Comparison of progeny relationships in a direct and an indirect line of* Strongyloides ratti *from September 22, 1937, to June 30, 1938*

	Direct Line	*Indirect Line*
No. of parasites	26	18
Serial generations	20–47	1–9
No. of cultures with one or more offspring	1463	921
Progeny		
Direct larvae	8344	2195
Indirect ♂ ♂	9	4232
Indirect ♀ ♀	1337	11255
Total progeny	9690	17682
Percentage of indirect progeny	13.9%	87.6%

about once in each thousand progeny observed. In the heterogonic line about one in every four progeny was a free-living male.

A difference of this magnitude requires no comment and re-emphasizes the difficulty in accounting for it on any basis other than that of inherent constitution in the parasites involved.

5. *Differences in Rate of Infection*

At the present time the heterogonic line has been passed serially through 12 parasitic generations. Of the 61 rats exposed to a single larva of indirect development 25 became infected and progeny were subsequently demonstrable in their feces. The infection rate thus obtained (41.0 per cent) is somewhat higher than that obtained in the homogonic line (26.2 per cent).

When the homogonic line had been passed through 34 serial generations it was reported (Graham, 1938a) that an infection rate of 25.6 per cent had been obtained from the exposure of 469 rats to a single larva each. At the 57th serial generation, 199 additional exposures have yielded patent infections at essentially the same rate (27.1 per cent). The consistency of the infection rate in the homogonic line is thus well established. However, it must be kept in mind that an infection rate of nearly 40 per cent was at one time observed in a consecutive series of 86 single larva exposures in this line (Graham, 1936, p. 82).

If the infection rate of 41 per cent in the heterogonic line (which is half again as great as that which obtained over the entire span of one homogonic line) is at or near its normal level, then this difference in the infection rate in the two lines is a mark of dissimilarity. Because of the essentially small number of exposures in the heterogonic line no practical value can be attached to a statistical comparison. If, however, the number of exposures in this line were tripled, with the present infection rate being either maintained or exceeded, then specific validity could be assigned to this difference by assuming that only one consequential variable was involved. Several other lines of *S. ratti* under study have shown infection rates greatly in excess of that in the heterogonic line. The consistency of these observations encourages the belief that the infectivity of filariform larvae of *S. ratti* depends on a constitutional factor which may vary in different lines of the parasite.

DISCUSSION

The results reported herein are clearly indicative of constitutional dissimilarity in the two lines of *S. ratti* under comparison. While the data have been analyzed principally in terms of two groups it is necessary to keep in mind the fact that each individual infection was composed of but a single parasite showing well-defined characteristics of its respective line. This use of single *S. ratti* as an infective agent has afforded an ideal basis for the comparison of "strains" or "varieties" of the parasite since it avoided the complications of, and led to conclusions not permitted by, mass infections.

It is noteworthy that *S. ratti* from the homogonic line produced *predominantly* progeny of direct development, while *S. ratti* from the heterogonic line produced *predominantly* progeny of indirect development. The establishment of this fact provides considerable clarification of the biology of this species.

Working with mass infections of *Strongyloides ratti*, *S. papillosus*, and *S. stercoralis*, Sandground (1926) sought to demonstrate "by the selection of infective larvae of the direct and indirect modes of origin, strains of the parasite that would exhibit *exclusively* direct or indirect development"

(italics mine). After passage of these mass infections through several generations with negative results, he concluded that his series of infections was "sufficiently extensive to prove that infections showing both types of development are not necessarily due to infection with larvae of two developmental types." Thus indirectly did he indicate that in mass infection experiments the "mode of larval reproduction" by itself is an inadequate basis for demonstrating "strains" of the parasite.

Essentially Sandground confirmed an experiment of Wilms (1897) with *S. stercoralis* in which exclusively filariform larvae of direct development were fed to a human subject. Subsequently progeny showing both modes of larval development were observed. Concerning this result Leichtenstern (1899) remarked, "Damit war die Hypothese oder vielmehr Vermutung von den zwei entwicklungsgeschichtlich völlig getrennten Varietäten erledigt." It is clear that Leichtenstern discarded entirely the idea that two "varieties" of *S. stercoralis* existed, propagating "ausschliesslich" by direct or indirect development.

Sandground (1926) posed the question: "To what an extent do 'varieties' of *S. stercoralis* occur in which the mode of development of the parasites is *exclusively* direct or indirect?" (italics mine). It is this insistence on the part of Leichtenstern and Sandground that a "strain" or "variety" must reproduce exclusively by one mode or the other which has confused the subject. Sandground's criticism of Leichtenstern's hypothesis concerning the origin and ancestral history of *S. stercoralis* in the temperate zone loses much of its force when one recognizes, as Sandground failed to do, that in at least some species of *Strongyloides* a "strain" does not necessarily show "exclusively" one or the other mode of development, but may show predominantly one or the other. Viewed in this light, the results of Sandground's selection experiments are not entirely disharmonious with the hypothesis which Leichtenstern (1899) stated thus: "Die *tropische Anguillula,* welche vorzugsweise die heterogene *Rhabditis*-generation erzeugt, hat sich in der *gemässigten* Zone im Laufe zahlloser Generationen immer mehr in eine *Anguillula* verwandelt, deren Embryonen von Haus aus zur *direkten* Larvenentwickelung bestimmt sind. Wenn die Larvenabkömmlinge der *Rhabditis stercoralis* die kontinuierliche Fortpflanzung der *Anguillula* unterhalten, so resultiert daraus eine *Anguillula,* deren Embryonen mehr und mehr die geschlechtliche Zwischengeneration begünstigen. So verhält es sich in den Tropen."

As a matter of fact the difference in the percentage of indirect progeny among total progeny observed in Sandground's *S. ratti* selection experiments is not substantial (7.3 per cent in the direct development series and 16.9 per cent in the indirect development series), though as he remarked, "infections induced through the agency of larvae of indirect development show a greater tendency to exhibit the heterogonic mode

of development." However, the percentage of indirect progeny in his indirect series was only a fifth of that obtained in the present line of single, heterogonically derived *S. ratti* (see Table 1). Discrepancies of this magnitude suggest that there may be many possible "strains", "varieties" or "lines" of *S. ratti* which are either indistinguishable or partially obscured in mass infections. Schuurmans-Stekhoven (1928) criticised Sandground's conclusions on this specific point by calling attention to the fact that a population "in which many factors are involved does not change its type if no selection of individuals for the breeding of clones takes place."

Evidence was adduced by Sandground (1926) purporting to show that parasitic females of *S. ratti* were syngonic. On the basis of his mass selection experiments in which he did not observe elimination of progeny of indirect development he concluded that "larvae of direct development bear in their genetic constitution the potentiality of giving rise to adults which will exhibit, to roughly the same degree, both modes of development among their offspring." In other words he inferred that the characteristics of *S. ratti* were conserved through the agency of larvae of direct development. Regarding this point Schuurmans-Stekhoven (1928) said, "I feel doubt that a hermaphroditic female is homozygotic in respect with the sex-determining gen or gens and if my assumption is correct, then certainly the offspring of a single hermaphroditic female represents a mixture of strains of different genetic constitution." There is no reason why such an assumption should be limited to sex-determining factors. Thus we are brought face to face with the question: Is *S. ratti* in the parasitic phase syngonic or parthenogenetic? While the question is not amenable to solution solely on the basis of evidence now in hand, it can be pointed out that from the *single S. ratti* of the homogonic line in the present study no evidence has been obtained which would indicate that their progeny represent "a mixture of strains." In serial passage through 57 generations, a remarkable constancy in characteristics has been noted. On the other hand it is definitely known that new and distinctive lines of *S. ratti* do arise from the indirect filariform larvae of the bisexual free-living generation. There is thus justification for believing that Schuurmans-Stekhoven's criticism may be valid and that Sandground's demonstration of syngonism in *S. ratti* may be less than adequate.

The results obtained from study of both the homogonic and heterogonic lines of singly established *S. ratti* are in essential harmony with Sandground's observations in serial mass infections. However, the differences noted between the two lines were far more decisive than any observed by Sandground in his study. It is to be especially noted that the fixation of characteristics in *S. ratti* by homogonic passage (a point subscribed to by both Sandground and the writer) seems incompatible with the idea of syngonism in the parasitic phase of the life cycle.

SUMMARY

1. Two lines of singly established *S. ratti* have been compared. One line was of homogonic origin, the other of heterogonic origin.

2. Several differences, referable to constitution, were noted between these two lines. Principal among these was the predominant production of progeny of indirect development by the parasites in the heterogonic line and the predominant production of larvae of direct development in the homogonic line.

3. The parasites of the heterogonic line reproduced at a more rapid rate and were reproductively shorter-lived than parasites of the homogonic line. The former also produced free-living males (indirect development) in large numbers, while homogonically derived parasites produced these males in very small numbers.

4. As the parasites in these two lines were compared under similar conditions and at the same time of year, it was concluded that the differences noted between them could not be attributed to factors in the fecal environment influencing the young rhabditiform larvae but were evidence of constitutional dissimilarity between the two lines.

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